Glycolysis: a potential therapeutic target in Spinocerebellar Ataxia Type 1

Javier Rafael Díaz García

Glicólisis: Potencial diana terapéutica en Ataxia Espinocerebellar Tipo 1

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Tesis Doctoral presentada por

Javier Rafael Díaz García

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Molecular and Human Genetics Department.
Baylor College of Medicine. Houston.

Jan and Dan Duncan Neurological Research Institute.
Texas Children's Hospital. Houston.

Biology Department,
Universidad Autónoma de Madrid. Madrid.

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Trabajo dirigido por
Dr. Juan Botas Rodríguez

Trabajo tutorizado por
Dra. Laura Torroja Fungairiño

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Si tuviera que elegir una vida, sin duda repetiría cada momento vivido para llegar al sitio donde me encuentro

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Desde el principio hasta el final…

Agradecer a mis padres por la educación que me han dado y por el apoyo constante a las decisiones que he ido tomando a lo largo de mi vida aunque eso supusiera irme a trabajar a miles de kilómetros.

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ABBREVIATIONS

1,3PG: 1,3-biphosphoglycerate
2-DG: 2-deoxy-D-glucose
2PG: 2-phosphoglycerate
3PG: 3-phosphoglycerate
Acetyl-CoA: acetyl-coenzyme A
AD: Alzheimer's Disease
ADP: Adenosine Diphosphate
ALDO: Aldolase
ALDOC: Aldolase C
AMPK: 5' AMP-Activated Protein Kinase
ATP: Adenosine Triphosphate
ATXN1: Ataxin-1
cDNA: complementary DNA
CIC: Capicua
CMA: Chaperone-Mediated Autophagy
CO2: Carbon dioxide
CR: Caloric Restriction
CT: Computed Tomography
DHAP: Dihydroxyacetone Phosphate
DNA: Deoxyribonucleic Acid
DRPLA: Dentatorubral-Pallidoluysian Atrophy
elav: Embryonic lethal abnormal vision
ENO: Enolase
ETS: Electron Transport Chain
F1,6BP: Fructose-1,6-Biphosphatase
F6P: Fructose-6-Phosphatase
FDG: Fludeoxyglucose
G6P: Glucose-6-Phosphatase
GADP: Glyceraldehyde 3-Phosphate
GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase
Gfi-1: Growth factor independent 1 transcription repressor
GFP: Green Fluorescent Protein
GLUT1: Glucose Transporter 1
GLUT2: Glucose Transporter 2
GLUT3: Glucose Transporter 3
GLUT4: Glucose Transporter 4
GMR: Glass Multimer Reporter
H2O: Hydrogen oxide, water
HD: Huntington Disease
HK: Hexokinase
Hsp70: Heat Shock 70kDa Protein
INSR: Insulin Receptor
IPA: Ingenuity Pathway Analysis
KI: Knock-In
LDH: Lactate Dehydrogenase
LOF: Loss Of Function
MAPK: Mitogen-Activated Protein Kinase
mRFP: monomeric Red Fluorescent Protein
MRI: Magnetic Resonance Imaging

MSK1: Mitogen- and Stress-activated protein Kinase

mTORC1: Mechanistic Target Of Rapamycin

NAD^+: Nicotinamide Adenine Dinucleotide Oxidized

NADH: Reduced Nicotinamide Adenine Dinucleotide

NLS: Nuclear Localization Signal

NMR: Nuclear Magnetic Resonance

nrv2: Nervana2

PC: Purkinje Cells

PCK1: Phosphoenolpyruvate Carboxykinase 1

Pcp2: Purkinje cell protein 2

PCR: Polymerase Chain Reaction

PD: Parkinson's Disease

PEP: Phosphoenolpyruvate

PET: Positron Emission Tomography

PFK: Phosphofructokinase

Pfkfb3: 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3

PGI: Phosphoglucose Isomerase

PGK: Phosphoglycerate Kinase

PGM: Phosphoglycerate Mutase

PK: Pyruvate Kinase

Pyr: Pyruvate

Q: Glutamine

RNA: Ribonucleic Acid

RNAi: Interference Ribonucleic Acid

RNAseq: RNA sequencing

S: Serine

SBMA: Spinal and Bulbar Muscular Atrophy

SCA1: Spinocerebellar Ataxia Type 1

SCAs: Spinocerebellar Ataxias

SEM: Scanning Electron Microscopy

Sens: Senseless

shRNA: small hairpin Ribonucleic Acid

SLC2A1: Solute Carrier Family 2 member 1

Slc2a13: Solute Carrier Family 2 member 13

SLC2A2: Solute Carrier Family 2 member 2

SLC2A3: Solute Carrier Family 2 member 3

SLC2A4: Solute Carrier Family 2 member 4

Slc5a1: Solute Carrier Family 5 member 1

TCA: Citric Acid Cycle

TG: Transgenic

TRiP: Ribonucleic Acid interference Bloomington collection

UAS: Upstream Activated Sequence

UPS:Ubiquitin–Proteasome System

WT: Wild-Type

YFP: Yellow Fluorescent Protein

ABSTRACT

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Spinocerebellar ataxia type 1 (SCA1) is one of nine Polyglutamine Diseases caused by CAG expansion in the coding region of the corresponding gene. In SCA1, CAG expanded repeats cause an abnormally long glutamine tract in ATXN1 protein and trigger a gain of function pathogenic mechanism that leads to a progressive neurodegenerative disorder. The brain regions primarily affected are the cerebellum and the brainstem. Previous studies reported that insulin sensitivity and insulin secretion are abnormal in SCA1 patients. We hypothesize that expression of mutant ATXN1 impairs glucose metabolism and use multiple approaches to test this hypothesis using mice and *Drosophila* disease models. Gene expression analyses in SCA1 mice and flies suggest glucose uptake deficiency in neuronal cells. Confirming these results, positron emission tomography imaging suggests lower glucose level in the cerebellum of SCA1 transgenic mice. Metabolomic (NMR) analyses in SCA1 fly neurons reveal a decrease in glucose metabolism metabolites. We carried out a genetic screen in *Drosophila* of all genes encoding glycolytic enzymes for potential modifier genes of SCA1 pathogenesis. We found that reducing the activity of glycolytic genes ameliorates neurodegeneration in the SCA1 *Drosophila* model. Further mechanistic studies indicate suppression of ATXN1-induced neurodegeneration is mediated by reduced steady-state levels of mutant ATXN1 protein. Together these data suggest that glucose metabolism impairments contribute to SCA1 pathogenesis and reveal new therapeutic approaches to decrease ATXN1 neurotoxicity.

RESUMEN

RESUMEN

La Ataxia Espinocerebellar Tipo 1 (SCA1) es una de las nueve Enfermedades Poliglutamínicas que se caracterizan por la expansión del triplete CAG en la región codificante del gen correspondiente. En SCA1, la expansión de las repeticiones de CAG causan un tracto de Glutaminas anormalmente largo en la proteína ATXN1 provocando una mutación de ganancia de función que provoca la aparición de neurodegeneration con carácter progresivo. Las regiones primariamente afectadas en SCA1 son el cerebelo y el troco cerebral. Estudios previos revelaron que pacientes con SCA1 desarrollan un aumento de resistencia a la insulina. Por lo cual, nosotros hipotetizamos que la expresión de ATXN1 mutante causa defectos en el metabolismo de la glucosa. Para corroborar esta hipótesis utilizamos diversas técnicas usando *Drosophila* y ratones como modelos de la enfermedad. Análisis de expresión genómica en moscas y ratones de SCA1 sugieren la deficiencia en el transporte de la glucosa en neuronas. Estos resultados fueron confirmados por tomografía de emisión de electrones en ratones transgénicos de SCA1. Análisis metabolómicos en moscas de SCA1 revelaron una reducción en metabolitos que participan en el metabolismo de la glucosa. Además llevamos a cabo un rastreo genético de genes que codifican enzimas glicolíticas para la búsqueda de modificadores genéticos. Este estudio reveló que la falta de función de las enzimas glicolíticas aminora la neurodegeneración reduciendo los niveles de la proteína mutante ATXN1. Todos estos datos sugieren que la patogenia de SCA1 causa defectos en el metabolismo de la glicólisis abriendo la puerta a nuevos avances terapéuticos para disminuir la neurotoxicidad producida por ATXN1.

INTRODUCTION

1. INTRODUCTION

1.1. POLYGLUTAMINE DISEASES

Spinocerebellar ataxia type 1 is one of the nine dominant inherited autosomal neurodegenerative diseases caused by the CAG expansion in the coding region of the gene encoding the protein ATXN1 (Zoghbi and Orr 1995). This group of neurological disorders, collectively known as Polyglutamine Diseases, includes Huntington disease (HD), Spinal and Bulbar Muscular Atrophy (SBMA), Dentatorubral-Pallidoluysian Atrophy (DRPLA), and the Spinocerebellar Ataxias (SCAs) 1, 2, 3, 6, 7 and 17 (Table 1). CAG expanded repeats cause an abnormally long glutamine tract in the disease-causing protein, and trigger a gain of function mutation of the protein that leads a progressive neurological disorder characteristic

PolyQ disease	Gene / Protein	Locus	Fly homolog gene	PolyQ expansion	
				Normal	Pathological
HD	HTT	4p16.3	Htt	6–35	36–121
SCA1	ATXN1	6p23	Atx-1	6–44	39–83
SCA2	ATXN2	12q24	Atx2	14–32	34–77
SCA3	ATXN3	14q24-q31	No homolog	12–40	62–86
SCA6	CACNA1A	19p13	Ca-alpha1D	4–18	21–30
SCA7	ATXN7	3p21–p12	No homolog	7–18	38–200
SCA17	TBP	6q27	Tbp	25–43	45–63
SBMA	AR	Xq11-q12	No homolog	6–36	38–62
DRPLA	ATN1	12p13	Gug	3–38	49–88

Table1. List of Polyglutamine Diseases. HD = Huntington's Disease; SCA = Spinocerebellar Ataxia; SBMA = Spinal Bulbar Muscular Atrophy; DRPLA = Dentatorubropallidoluysian Atrophy; HTT (Htt) = Huntingtin; ATXN1/2/3/7 = Ataxin-1/2/3/7; CACNA1A = Calcium Channel, Voltage-Dependent, P/Q Type, Alpha 1A Subunit; TBP (Tbp) = TATA-binding protein; AR = Androgen receptor; ATN1 = atrophin-1; Atx-1 = Ataxin 1; Atx2 = Ataxin 2; Ca-alpha1D = Ca^{2+}-channel protein α_1 subunit D; Gug = Grunge. (Bauer and Nukina 2009)

of these diseases (Gatchel 2005). Although the number of CAG triplet repeats necessary to trigger pathogenesis differs between polyQ disorders (Table 1), the length of the repeat expansion positively correlates with disease severity and age of onset. (H. Orr 2001, Gatchel 2005).

Polyglutamine proteins are widely expressed in the nervous system and it is still controversial why there is differential neuronal vulnerability for each polyQ disease. For example in HD the neurodegeneration is primarily observed in the striatum (Ginovart 1997), whereas SCA's are characterized by cerebello-olivary atrophy (Klockgether 2008).

A prominent neuropathological feature observed in most of these disorders is the age-dependent formation of cytoplasmic and/or intranuclear accumulation of aggregated proteins inside neurons that contain the polyQ protein and other proteins involved in the ubiquitin–proteasome and molecular chaperone systems (Ross 1997, DiFiglia 1997). Nuclear aggregates can abnormally interact with nuclear proteins and lead to gene transcriptional dysregulation and neuropathology (Li 2007). Cytoplasmic accumulation can alter axonal transport disrupting synaptic function and glutamate release (Li 2003).

Although these studies suggested that both nuclear and cytoplasmic aggregates of polyQ proteins cause pathogenesis, these aggregates may also protect by sequestering toxic oligomeric species (Saudou 1998, Lasagna-Reeves 2015).

The polyQ inclusions usually appear in the affected brain regions, thereby in the case of SCA1 intracellular aggregations are observed largely in the Purkinje cells (PC) and granular cells.

1.2. ATAXIA SPINOCEREBELLAR TYPE 1

SCA1 is a neurodegenerative disorder characterized by progressive dysfunction that affects Purkinje cells in the cerebellum, cells of the inferior olive in the cerebellum cortex and neurons within brainstem cranial nerve nuclei (Zoghbi and Orr 1995) (Fig1). SCA1 is caused by the CAG expansion in the *ATXN1* gene, which is localized in the short arm of chromosome 6 (Zoghbi and Orr 1995). The gene was cloned in 1993 by Dr. Huda Zoghbi (Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States) and Dr. Harry Orr (Institute for Translational Neuroscience, University of Minnesota, Minnesota, United States) and found to encode a novel protein designated ATXN1 (ataxin-1) (Orr 1993). Approximately 1-2 in 100,000 people will develop SCA1, however the frequency varies considerably based on geographical location and ethnic background (data from National Ataxia Foundation).

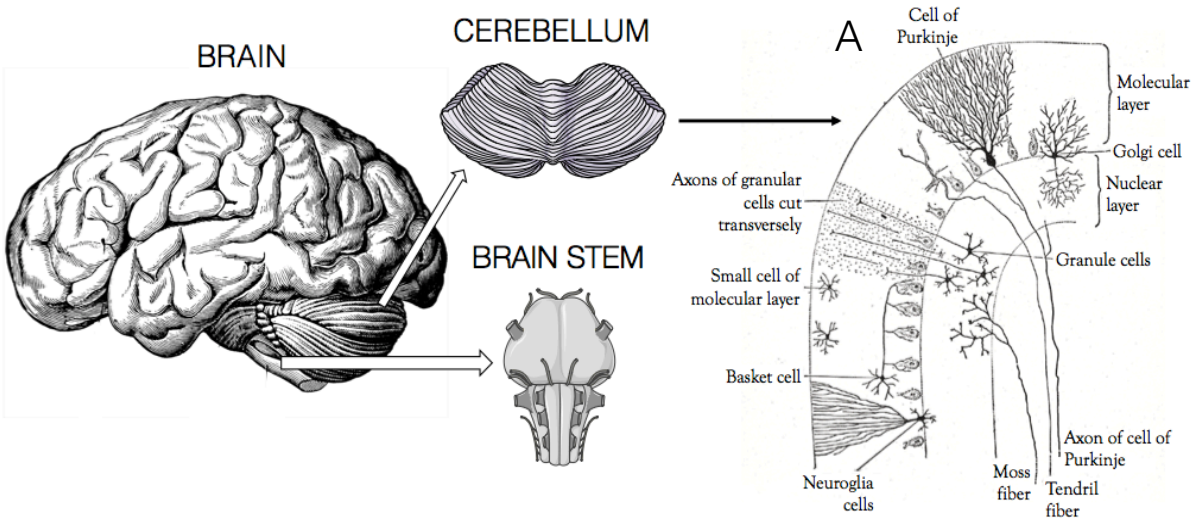


Figure 1. Brain affected areas in SCA1 disease and cerebellum histology. Cerebellum and brain stem are the mainly affected areas in SCA1 patients. Cells of the inferior olive and Purkinje cells are localized in the cerebellum. Cranial nerve nucleus is situated in the brainstem. A. Transverse section of a cerebellar folium. Purkinje cells dysfunction is crucial for SCA1 pathology. Cerebellum histology drawing was published in "Anatomy of the Human Body" (1918) by Henry Vandyke Carter and Henry Gray.

The cerebellum is a region of the brain (Fig 1) that has an important role in coordination, precision, and accurate timing of body movement; therefore it is crucial for a precise motor control. It is also involved in some cognitive function such as attention and language. The cranial nerve nucleus, which is a collection of neurons in the brain stem (Fig. 1), also plays an important role in motor activity. Thus since SCA1 causes neurodegeneration of the cerebellum and cranial nerve nucleus, most clinical features are related to the motor control. Among these features we find ataxia, spasticity, ophthalmoparesis, slow saccades, and cognitive impairment such as impairment in visual-spatial perception and attention (Zoghbi and Orr, 1995, Ma 2014). With the progression of the disease other symptoms emerge such as dystonia, tongue atrophy, dysphagia, dysphonia, deep sensory loss, etc (Zoghbi and Orr, 1995). At present there is no treatment for SCA1. The medication is indicated to alleviate the symptoms of the disease.

ATXN1 normal alleles contain between 6 and 39 CAG repeats located within exon 8 (Orr 1993). The pathogenesis appears when the CAG expansion of the glutamine-encoding CAG tract exceeds 40 repeats (Chung 1993). The typical age of onset is in the third or fourth decade with variations depending on repeat length. Anticipation refes to the observation that CAG repeats can expand further during male germline transmission, causing earlier onset and increased severity of the disease in successive generations (Zoghbi and Orr 2009).

Mutant *ATXN1* tends to accumulate in the nucleus to form insoluble aggregates (Cummings 1998). The primary pathogenic role of these structures is still unclear since neurons with inclusions are not always the most vulnerable to death. Immunohistochemical staining of aggregates reveals components of the proteasome system and ubiquitin, which are involved in protein turnover. These findings suggest that impairments in proteasome function likely contribute to the pathogenesis of the disease (Cummings 1998, Taroni 2004) (Fig 2).

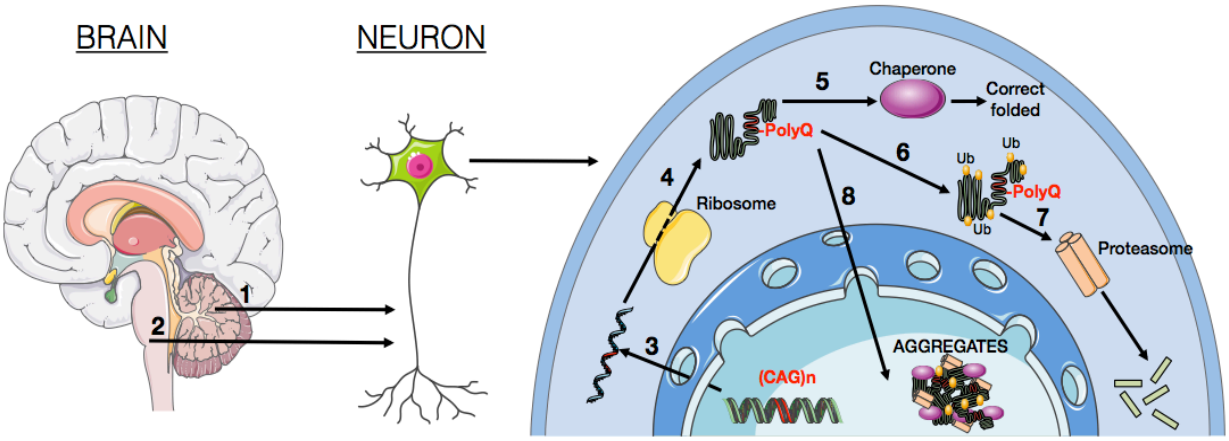


Figure 2. Proposed pathogenic mechanisms of ATXN1. Cerebellum and brainstem are the major areas affected in SCA1 (**1**,**2**). The mutant ATXN1 gene contains expanded CAG repeats which are transcribed (**3**) and translated (**4**) into long polyglutamine tracks. Due to the polyQ expansion, ATXN1 acquires an aberrant conformation, which can be corrected by molecular chaperones (**5**). Misfolded ATXN1 protein may be ubiquitinated (Ub) (**6**) and degraded via the proteasome (**7**). ATXN1 protein is imported into the nucleus (**8**) where it potentially form aggregates. In addition to ATXN1 protein, aggregates contain transcription factors, ubiquitin, and other proteasome components. (Adapted from Taroni, 2004)

1.2.1. Ataxin-1 Protein (ATXN1)

Genetic and molecular evidences indicate that in SCA1, neurodegeneration is mainly caused by a gain-of-function mechanism in the ATXN1 protein. Evidences includes null *Atxn1* mice, which are viable and fertile, and do not develop ataxia or a neurodegeneration phenotype (Matilla 1998). Also it is important to point that abnormally high levels of wild-type ATXN1 can cause degenerative phenotypes comparable to those caused by the expression of the expanded protein (Fernandez-Funez, 2000). However other data suggest that partial loss of ATXN1 function might contribute to pathogenesis. For example studies in mice show that deletion of the wild type *Atxn1* gene enhances SCA1 pathogenesis, and an

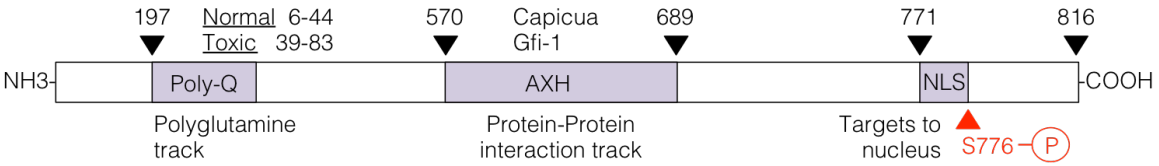


Figure 3. Protein domains critical for ATXN1 function and SCA1 pathogenesis. The functional domains are indicated as purple boxes. This protein corresponds to ATXN1 wild-type allele with 816 amino acids and 30 glutamine repeats. Wild-type ATXN1 contains 6-40 glutamines, while mutant ATXN1 may contain from 39 to 83 glutamines. The AXH domain has the ability to interact with other proteins such as Capicua and Gfi-1. The NLS domain targets ATXN1 to the nucleus. Finally, the residue of the NLS, Ser^{776}, is an important site of phosphorylation in ATXN1. (Adapted from Zoghbi and Orr, 2008)

increase of Ataxin 1-Like gene, which is a conserved paralog of ATXN1, ameliorates the neurodegeneration. (Crespo-Barreto 2010)

The polyQ expansion is necessary but not sufficient to cause toxic events (Fernandez-Funez, 2000). Studies revealed important domains in ATXN1 protein that are critical for SCA1 pathogenesis including the AXH domain, the nuclear localization signal (NLS),and a single serine residue localized at position 776 (S776) (Fig. 3). In fact, the overexpression of polyglutamine-expanded ATXN1 lacking any of these domains ameliorates Purkinje cell degeneration reducing pathogenesis events in mice (Klement 1998, Emamian 2003, Tsuda 2005).

The AXH domain is a 130 amino acids-long protein motif, which forms an oligonucleotide-binding fold and is important for dimerization of ATXN1, for protein-protein interaction and RNA binding (de Chiara 2003). Proteins already identified that bind to ATXN1 via interactions with its AXH domain include Capicua (CIC), which is a transcriptional repressor whose repressing activity and levels are modulated by ATXN1 (Lam 2006). Also the zinc-finger transcription factor Senseless (Sens) in *Drosophila* and its mammalian homolog Gfi-1 interact with

ATXN1 through the AXH domain and this interaction may contribute to the selective vulnerability of Purkinje cells observed in SCA1 (Tsuda 2005).

ATXN1 contains a nuclear localization signal (NLS) close to its C-terminus. To study the role of the NLS, mice were generated expressing ATXN1 with 82 CAG repeats and a mutated NLS. This mutant ATXN1^{82Q} accumulated within the cytoplasm of Purkinje cells and did not form aggregates. In addition PC from these mice did not show cell histopathology such as nuclear inclusions or degeneration. Furthermore they did not display an abnormal neurological phenotype (Klement 1998).

Phosphorylation is an important mechanism to regulate protein activity. Examination of all the ATXN1 residues susceptible to be phosphorylated lead to the finding that serine 776 (S776) was phosphorylated (Emamian 2003). To study the effect of this phosphorylation, S776 was replaced with a residue of alanine to prevent phosphorylation and its effect was studied in cell and mouse SCA1 models. In both cases the pathogenesis was considerably reduced, indicating that S776, and possibly protein conformation, have key roles in the toxicity generated by mutant SCA1 (Emamian 2003).

1.3. ANIMAL MODELS OF NEURODEGENERATIVE DISEASES

Neurodegenerative disorders are characterized by a gradual loss of the neuronal cell population. This leads to cognitive, behavioral and/or physical impairments. The limitation of human trials is the difficulty in analyzing the cellular pathology for greater understanding of the disease. Thus, animal models for different neurodegenerative diseases models have been developed to reproduce the largest possible number of features of these disorders in simpler systems.

1.3.1. *Drosophila* model

Drosophila has proved to be an excellent model for genetic studies in human neurological disorders due to several advantages:

- Enables the expression of human genes, both in mutant and wild-type forms.
- Allows study of gain or loss of function mutations in human homolog genes.
- Appropriate for large-scale genetic screening.
- Simpler fly genome compared to the complex human genomic organization.
- Lack of many redundant genes in flies.
- Availability of versatile genetic manipulation procedures.
- Over 75% of human genes are conserved in *Drosophila* and importantly, cell biology mechanisms are highly conserved such as neuronal connectivity, regulation of gene expression, cell signaling and cell death (Rubin 2000).

The GAL4/UAS system (Fig 4) (Brand and Perrimon 1993) allows to control of transgene expression levels and targeting to different cell types, including specific populations of neurons. This system also provides a temporal targeted gene expression strategy in *Drosophila* (Brand and Perrimon 1993).

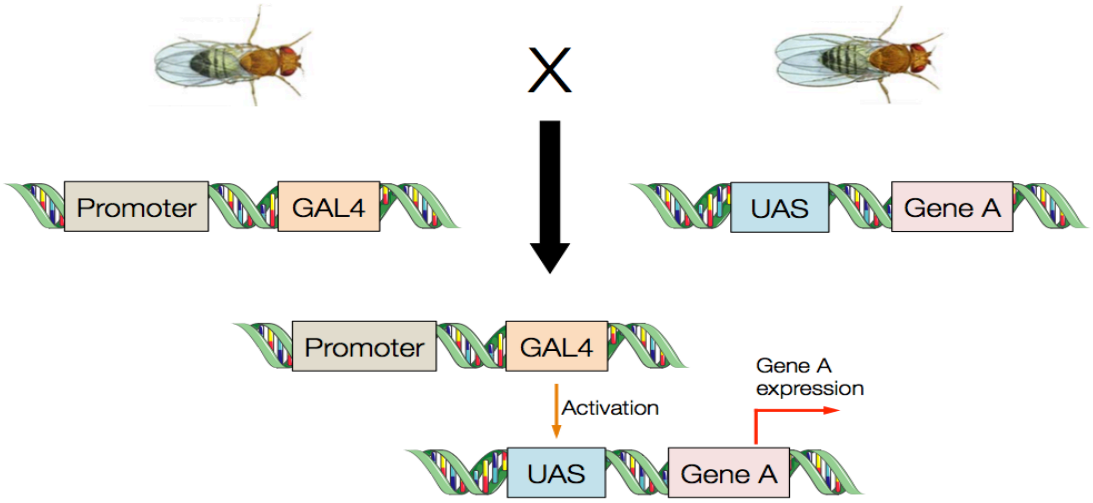


Figure 4. UAS-GAL system. This binary system is used to target gene expression in a specific cell population such as neurons. The gene to be expressed is placed downstream of the yeast upstream activating sequence (UAS) element. In the absence of the yeast GAL4 transcription factor, the UAS-GeneA transgene remains silent. To activate transcription of the UAS-GeneA, flies that contain this construct (UAS-GeneA) are mated with flies carrying the Promoter-GAL4 driver. The resulting F1 progeny larvae and adults express the GeneA (Brand and Perrimon, 1993).

1.3.1.1. ATXN1^{82Q} *Drosophila* model

To carry out genetic studies of SCA1, a transgenic *Drosophila* was created by Dr. Juan Botas (Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA) laboratory 15 years ago (Fernandez-Funez 2000). This SCA1 fly model contains an insertion of the full-length human *ATXN1* gene with 82 CAG repeats, which encodes an ATXN1 protein with a 82 aa-long polyglutamine tract. The level of the transgene expression is controlled using the UAS/GAL4 system (Fig. 3).

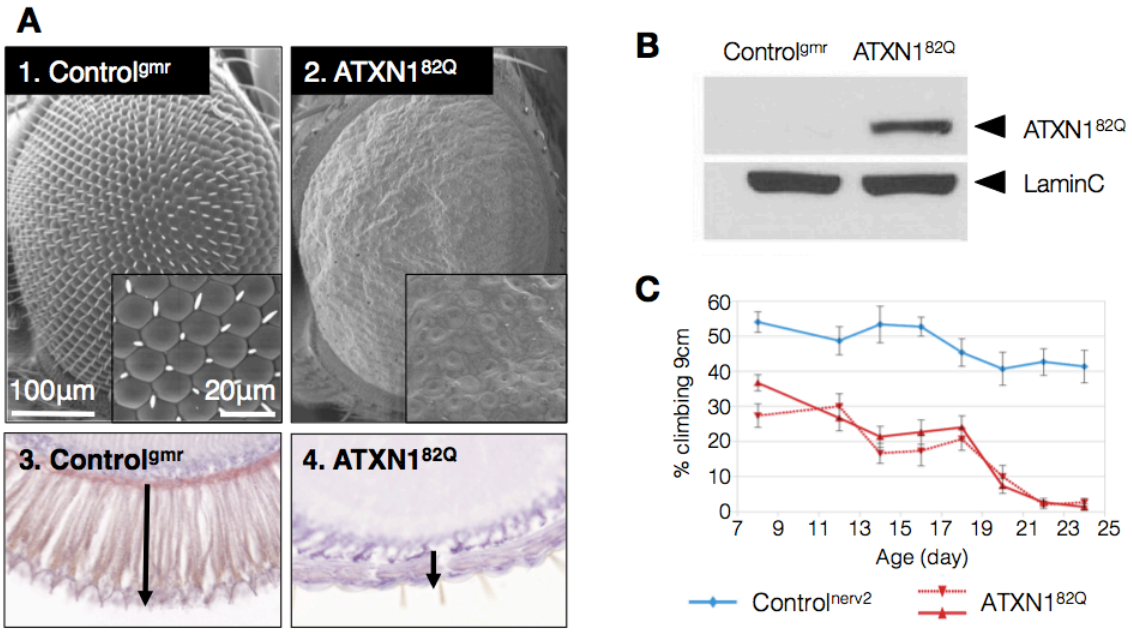


Figure 5. *Drosophila* ATXN1 [82Q] model. (A) Strong eye phenotypes caused by SCA1 overexpression. 1-2, scanning electron microscope (SEM) eye images of gmr–GAL4/+ control (1), and UAS: SCA1 82Q/+; gmr–GAL4/+ (2). Insets correspond to magnification of ommatidia field. 3-4, Paraffin sections (hematoxylin stain) through the retinas of gmr–GAL4/+ control eyes (3), and UAS: SCA1 [82Q]/+; gmr–GAL4/+ (4) transgenic flies at the same magnification. Flies were raised at 28.5 °C. Degeneration of the retina (arrow) is severe in ATXN1[82Q]-expressing flies. (B) Western blot of head extract shows the presence of ATXN1 in this *Drosophila* SCA1 model. (LaminC: loading control). (C) Modification of UAS: SCA1 [82Q]/+; nrv2–GAL4/+ neuronal phenotype in motor performance assay. Quantification of the climbing performance of flies as a function of age. The nervana-GAL4 (nrv2) driver was used to direct expression of the mutant proteins to the nervous system Flies were raised at 28.5 °C.

To serve as a good disease model, the transgenic flies must recapitulate the main pathogenic features of the disease in humans. These flies exhibited the main characteristics of the SCA1 features in humans such as neurotoxicity, the pattern of SCA1 progression and the presence of nuclear inclusions. The expression of the ATXN1^{82Q} transgene in the nervous system causes a progressive reduction of cell bodies and axonal projections. In addition, a variety of cell types, including central nervous system neurons and photoreceptors, show nuclear aggregates. These nuclear aggregates contain ubiquitin, proteasome and Hsp70 chaperone. The transgenic flies also recapitulate another important feature of SCA1 pathogenesis,

which is the positive correlation between the severity and early disease onset with the number of CAG repeats expansion. (Fernandez-Funez 2000).

These flies were the first model used in a genetic screen for modifiers (suppressors and enhancers) of a neurodegenerative disease. The screen identified potential modifiers such as genes involved in ubiquitin-proteolytic pathways, protein folding/heat-shock response and genes that encode proteins containing RNA-binding domains (Fernandez-Funez 2000).

In this study, we used SCA1 transgenic flies with one of the two different constructs. In the first construct, the expression of $ATXN1^{82Q}$ is driven by the eye specific driver, gmr-GAL4 to cause retina degeneration (Fig 4). In the other construct, the expression of $ATXN1^{82Q}$ is driven by the pan neuronal driver nrv2. To study the progressive neurodegeneration caused by $ATXN1^{82Q}$, we performed motor behavioral test, where we measured the ability of the fly to climb in successive time points (Fig. 5).

A couple of years ago, a cross-species strategy was developed: combining forward genetic screens that targeted the levels of mutant human ATXN1 in the SCA1 *Drosophila* model and a human cell model of SCA1. The cells were generated from a human medulloblastoma-derived cell line, and contain a transgene encoding a CAG-expanded ATXN1 fused with monomeric red fluorescent protein (mRFP–$ATXN1^{82Q}$). Placing an internal ribosomal entry site (IRES) followed by yellow fluorescent protein (YFP)-encoding sequence downstream of the ATXN1 fusion sequence, allowed to detect changes in the $ATXN1^{82Q}$ protein levels using the ratio of mRFP to YFP fluorescence as read-outs. We found that the downregulation of numerous components of the RAS-MAPK-MSK1 pathway suppresses neurodegeneration in SCA1 flies, and decreases ATXN1 levels in both cells and flies. Importantly we carried out a drug trial assay revealing new therapeutic approaches to decrease ATXN1 pathogenic (Park 2013).

1.3.2. *Mus musculus* model

Mouse is a powerful model to study the mechanisms of neurological diseases and for testing potential therapies. This is due to the high conservation of genes between human and mouse. In addition, the ability to manipulate the mouse genome has been rapidly growing (Cathleen 2014).

Technical approaches to generate neurodegenerative mouse models include reverse genetics (Capecchi 1989), transgenic mice (Palmiter 1985), exogenous transposon-transposase system (Largaespada 2009), mutagenesis using mutagen agents (Justice 1999), and introducing interfering RNAs (RNAi) (Tiscornia 2003). These techniques allow generating different types of mouse models of diseases (Fig 6):

- Knockout: an endogenous gene is inactivated by replacing or disrupting it with an artificial piece of DNA, using a mutagen or by gene editing technologies.
- Knockdown: gene expression is downregulated, for example by RNAi.
- Transgenic: insertion of an exogenous gene into the genome.
- Knock-in: insertion of a protein coding cDNA sequence in a specific locus in the mice genome. The difference between knock-in and transgenic technology is that a knock-in involves a gene inserted into a specific locus. . In principle, knock-in mice are the best approach to generate an accurate genetic model, because they may guarantee accurate spatial and temporal expression patterns at endogenous levels.

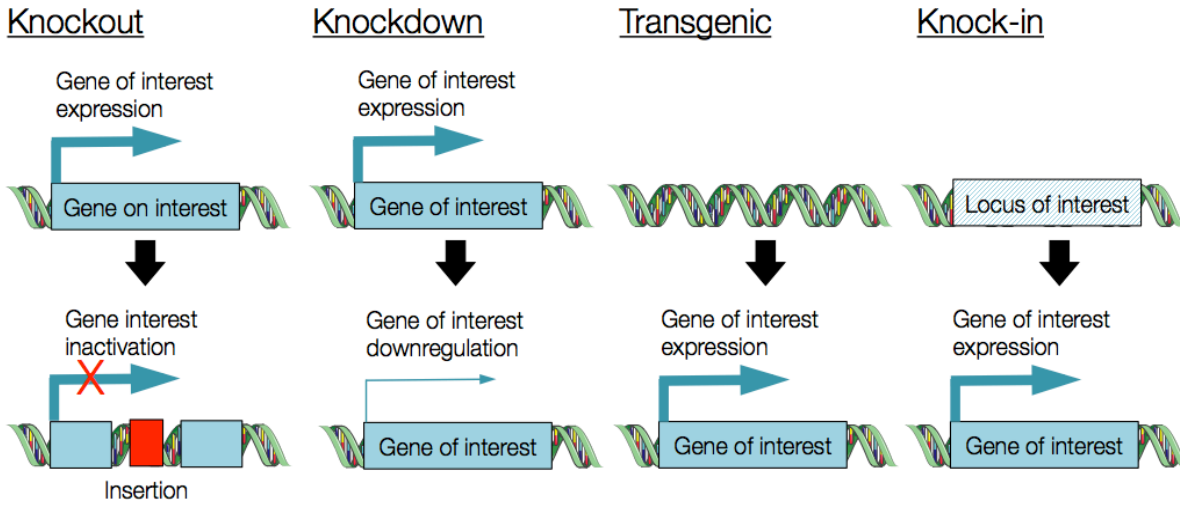


Figure 6. Types of mutated mice. Knockout: Inactivation of an existing gene by replacing it or disrupting it with an artificial piece of DNA. Knockdown: Reduction of the expression of a gene through genetic modification or by treatment with reagents such as RNAi. Transgenic: Insertion of an exogenous gene into the genome. Knock-in: insertion of a protein coding cDNA sequence at a particular locus.

1.3.2.1. SCA1 transgenic mouse model

Transgenic mice were generated to gain insight of the SCA1 pathogenesis. They express the mutant human *ATXN1* gene with an expanded CAG tract with 82 repeats. This transgenic mouse strain is called B05. As a control (called A02), a normal human ATXN1 gene with 30 CAG repeats was inserted into the mouse genome (Burright 1995). Expression of these transgenes was directed to the cerebellar Purkinje cells by the driver Pcp2 (Vandaele 1991), since these cells are the primary site of SCA1 cerebellar pathology.

To examine intergenerational stability of CAG repeats, 254 transgenic animals were studied and no changes in repeat size were observed (Burright 1995). Transgene expression levels were approximately 100-fold higher than endogenous

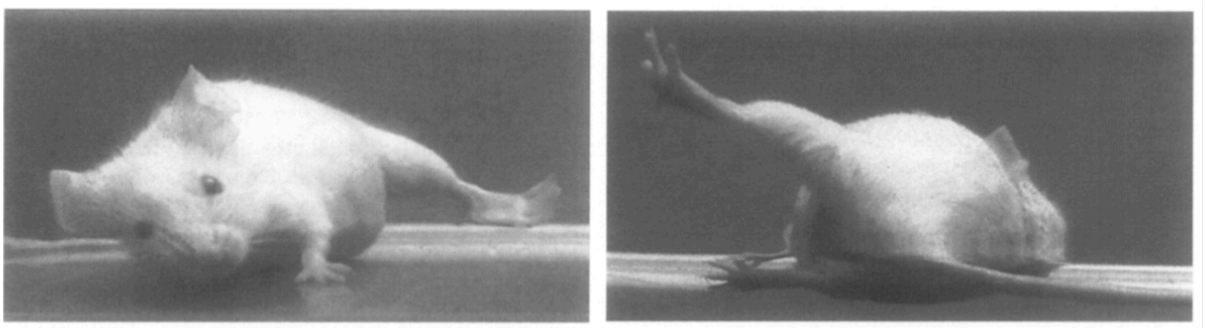


Figure 7. B05 transgenic mice ataxia phenotype. B05 (+/-) mice display clear signs of ataxia by falling to one side when walking at the age of 30 weeks. (Burright 1995)

level of ATXN1 RNAs measured by reverse transcriptase-PCR reaction (Burright 1995).

B05 mice exhibited motor performance abnormities starting at five weeks of age (Fig. 8), as assayed by a set of motor tests, which included footprint patterns assay, rotating rod, bar cross apparatus and open field test (Clark, 1997). Heterozygous B05 mice developed ataxia at twelve weeks of age (Fig. 7) and displayed an abnormal neurological phenotype, assessed by home cage behavior (Burright 1995, Clark 1997).

The pathogenic events observed in Purkinje cells include cytoplasmic vacuolization (day 25), simplification of the dendritic tree (six weeks), cell loss (twelve weeks) shrinkage of the molecular layer (fifteen weeks) and frequent heterotopia (fifteen weeks) (Clark 1997) (Fig. 8). All the phenotypes and pathology progressed with age (Clark 1997)

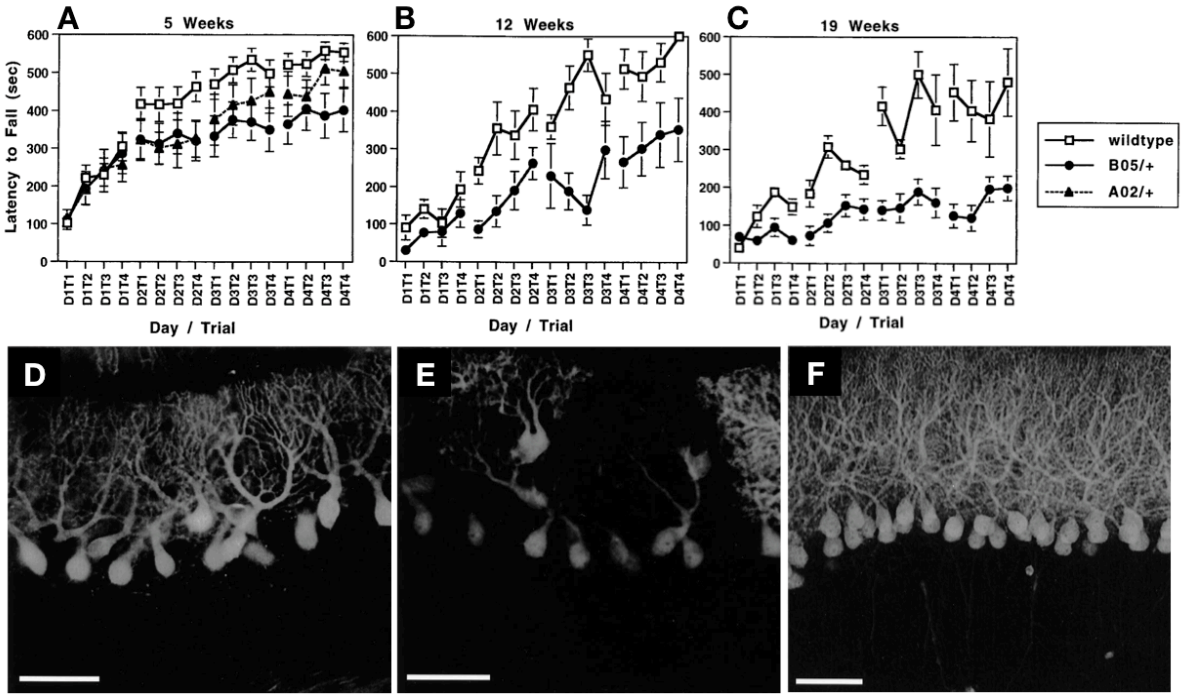


Figure 8. Behavioral phenotype and Purkinje cell (PC) histopathology in B05 transgenic mice. (A-C), rotating rod performance. (A), five-week old. (B), twelve-week-old. (C), nineteen-week-old. (A-C), B05/+ animals showed performance improvement. (D-F), immunohistochemical staining of cerebellar sections of SCA1 transgenic mice with calbindin. D, B05/+, 15 weeks old, animals showed shrinkage of the molecular layer and many PCs with atrophic dendritic morphology. Occasional PCs are located heterotopically in the molecular layer. E, B05/+ animal at 27 weeks old with increased severity compared to D. In addition, occasional larger hypertrophic PCs were apparent. F, A02/+ animal at 1 year old showed normal PCs number and dendritic arborization. Scale bars: 30 mm in D–F. B05/+ with 82 repeats. A02/+ with 30 repeats. Adapted from (Clark 1997)

1.3.2.2. SCA1 knock-in mouse model

In addition to the B05 mice, which showed a phenotype associated with dysfunctional Purkinje cells, SCA1 knock-in mice carrying 154 CAG repeats into the endogenous mouse ATXN1 locus were also generated (Watase 2002). SCA1 knock-in mice exhibited characteristic pathological features associated with SCA1 in humans such as cognitive impairment and shorter lifespan (Watase 2002)

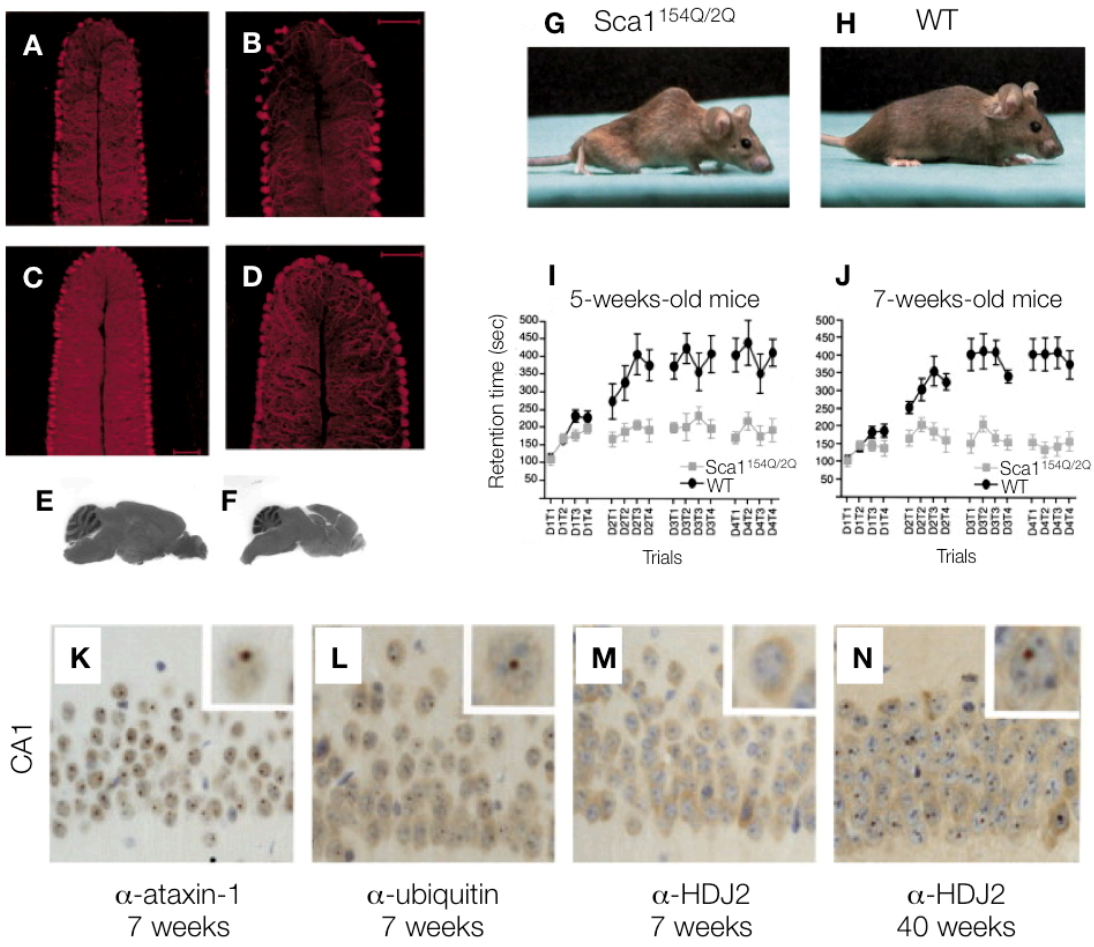


Figure 9. Phenotypes and pathology of knock-in SCA1$^{154Q/2Q}$ mice. (A–D), Immunofluorescence confocal microscopy images of 34 weeks old Sca1$^{154Q/2Q}$ cerebellums. Mutant animals (A and B) show altered cerebellar morphology compared to wild type mice (C and D). Staining with α-calbindin. Scale bars: 100 mm. (E and F), sagittal sections from the wild type (E) and mutant (F) brains (40 weeks old) showed brain atrophy in Sca1$^{154Q/2Q}$ mice. G and H, representative images of 28 weeks old mutant and wild-type mice. Mutant mice (G) show severe kyphosis (curvature of the spine). I and J, rotating rod performance. Sca1$^{154Q/2Q}$ animals showed impaired performance at five week-old (I) and seven-weeks-old (J). (K-N), CA1 hippocampal neurons images from mutant animals. Nuclear inclusions were positive for Atxn-1 (K) and ubiquitin (L) in seven week-old, but not for HDJ2 chaperone (M) seven week-old mice. In a 40 weeks old, most of the CA1 neurons are positive for HDJ-2 (N). Insets show individual CA1 neurons at higher magnification. Original magnification for each image was 200X. Adapted from (Watase 2002).

These mice show pathological phenotypes that included: growth retardation (eight weeks old); muscle wasting, ataxia, and abnormal gait (twenty week-old); impaired performance in rotating rod (five and seven weeks old) (Fig. 9); severe kyphosis (curvature of the spine) accompanied by atrophy of lower limb muscles (30 weeks old) (Fig 9) and premature death (35 and 45 weeks old). They also show spatial memorial deficits assessed using the Morris water maze at five to seven weeks old (Watase 2002).

Neuropathology studies revealed brain atrophy (Fig. 9), and redaction of Purkinje cell dendritic arbors with approximately 50% less fluorescence than control animals at 19 weeks old (Fig. 9). Nuclear inclusions of ATXN1 were observed starting at six weeks of age in mutant animal brains in multiple neuronal populations (Fig 9). In addition, the inclusions were positive for ubiquitin (7 weeks old) and chaperons (40 weeks old) (Watase 2002) (Fig 9).

1.4. GLUCOSE METABOLISM

Glucose is a monosaccharide with the molecular formula $C_6H_{12}O_6$. The name "glucose" comes from the Greek word gleûkos, meaning "sweet wine, must". With six carbon atoms, it is classified as a hexose, a sub-category of monosaccharides.

Cells use glucose as a primary source of energy through aerobic respiration, generating biochemical energy in the form of adenosine triphosphate (ATP) (Berg 2002). Aerobic respiration is a multi-step of catabolic reactions consisting of a series of biochemical steps, which include glycolysis, oxidative decarboxylation of pyruvate, citric acid cycle and oxidative phosphorylation. Glucose is the most important starting substrate for these reactions, although other nutrients can also be used. These compounds are catalyzed to the final product of form CO_2 and H_2O, obtaining energy mostly in the form of ATP (Berg 2002).

Photoautotrophic organisms like plants synthesize glucose by photosynthesis using water and carbon dioxide. However, heterotrophic organisms are unable to perform this process and they take glucose from other organisms. Glucose can also be obtained from other sugars, such as fructose and galactose. Thus, Glucose is one of the three dietary monosaccharides, along with fructose and galactose, which are absorbed directly into the bloodstream during digestion. Another way to obtain glucose is by synthesis from non glucidic molecules, a process known as gluconeogenesis. There are certain glucose precursor molecules, such as lactate, amino acids, oxaloacetate and glycerol (Eisenstein 1967).

1.4.1. Glucose uptake and glycolysis

Insulin is a polypeptide hormone produced and secreted by β-cells of the islets of Langerhans in the pancreas. It was discovered with the contributions of Frederick Grant Banting, Charles Best, James Collip, and J.J.R. Macleod, in 1922. Insulin binds to the insulin receptor (INSR) inducing translocation of glucose transporters to the plasma membrane and allowing glucose transport to the cell (Schulingkamp 2000) (Fig 10). Cell sensitivity impairment to insulin reduces glucose uptake, which results increase in blood glucose (Wilcox 2005). This abnormal event is known as insulin resistance and it is characteristic in type 2 diabetes (Lin 2010). A previous report showed insulin sensitivity and insulin secretion impairment in normoglycemic SCA1 patients. In this study, twelve SCA1 untreated and unrelated patients were analyzed revealing insulin secretion impairment, as well as simultaneous decrease in insulin sensitivity (Lalić 2010).

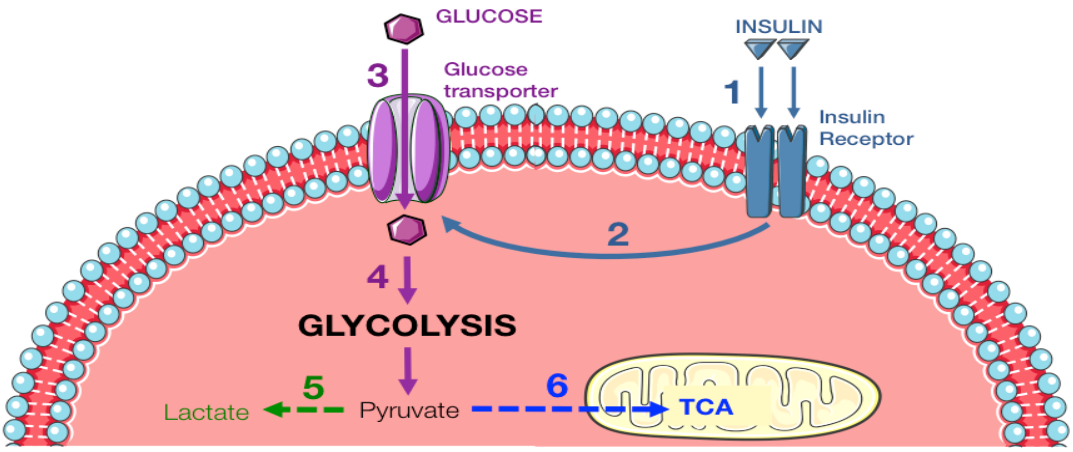


Figure 10. Effect of insulin on glucose uptake and glucose metabolism. Insulin molecule(s) binds to the insulin receptor (INSR) (1), which is activated. Activation of INSR can result in translocation of glucose transporter (Glut) to the membrane surface (2) and mediation of glucose entry into the cell (3). Glucose is metabolized to pyruvate by glycolysis (4). Pyruvate can supply energy to living cells through the TCA when oxygen is present (aerobic respiration) in the mitochondria (5). Alternatively pyruvate ferments to produce lactate when oxygen is absent (fermentation) in the cytoplasm (6). TCA, citric acid cycle, also known as the tricarboxylic acid. (Schulingkamp 2000), (Bolaños 2010) (Wilcox 2005)

The insulin receptor (INSR) is a member of the ligand-activated receptor and tyrosine kinase family of transmembrane signaling proteins. INSR is a dimer consisting of two α subunits that contain the site for insulin binding, and two β units where the tyrosine kinase domain is localized. The principal physiological role of the INSR seems to be metabolic regulation, whereas all other receptor tyrosine kinases are involved in other pathways such as regulation of cell growth and differentiation (Lee 1994).

The activation of INSR triggers the translocation of glucose transporter to the membrane that mediates the facilitated influx of glucose (Schulingkamp 2000) (Fig 10). Most glucose transporters belong to the GLUT (SLC2A) family of lipid bilayer membrane transport proteins. In humans, fourteen GLUT proteins have been identified. They can transport other metabolites besides glucose, such as fructose, myoinositol, vitamins and urate (Thorens 2010). GLUT1-GLUT4 transporters are the well-characterized glucose transporter isoforms.

- GLUT1 (SLC2A1): It is expressed at highest levels in the endothelial cells of blood–brain barrier tissue in human adults, supplying glucose to the cells of the central nervous system (Takata 1990). GLUT1 is normally upregulated in high sugar demanding tissues such as cancer tumors (Ganapathy 2009).
- GLUT2 (SLC2A2): It is the principal transporter to transfer glucose between liver and blood. It is also expressed in intestine and pancreas (Thorens 1992).
- GLUT3 (SLC2A3): It is the most important glucose transporter in neuronal tissue. It is localized in both dendrites and axons. Its expression level in different regions of the brain correlates with regional cerebral glucose utilization (Simpson 2008).
- GLUT4 (SLC2A4): The principal glucose transporter protein that mediates glucose uptake after the food ingestion is GLUT4 (Huang 2007). It is mainly present in skeletal, adipose and cardiac muscle, but it also localizes in some brain regions as hippocampus (Huang 2007, Patel 2014).

1.4.2. Glycolysis

The elucidation of the glycolytic pathway started in 1860 with Louis Pasteur observing that microorganisms were responsible for fermentation. By 1940 the complete glycolytic pathway was elucidated by the combined efforts of several scientists including Otto Fritz Meyerhof (Kresge 2005).

Glycolysis (from glycose-, an older term for glucose, and -lysis degradation) is the metabolic pathway that oxidizes glucose into pyruvate for the energy obtaining. The energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide) (Bolaños 2010, Pelicano 2006) (Fig 11).

Glycolysis process occurs in the cytoplasm and does not use molecular oxygen for any of its reactions. However, glycolysis products can follow different paths to be processed. When molecular oxygen is used in the disposal of the products of glycolysis, the process is usually said to be aerobic, while if the disposal does not use oxygen, the process is referred to be anaerobic (Bolaños 2010, Li 2015).

Glycolysis includes 10 reactions and can be divided in two phases (Fig 11):

- The Preparatory Phase or Investment Phase: In this stage ATP is consumed and includes the first five reactions.
    1. The first reaction is the phosphorylation of glucose to form glucose 6-phosphate (G6P) catalyzed by a family of isoenzymes known as hexokinases (HKs). In mammalian four isoenzymes of hexokinase have been identified. HKs are allosterically inhibited by product accumulation (G-6-P). This reaction consumes ATP and is irreversible. This step helps to keep low glucose levels into the cell,

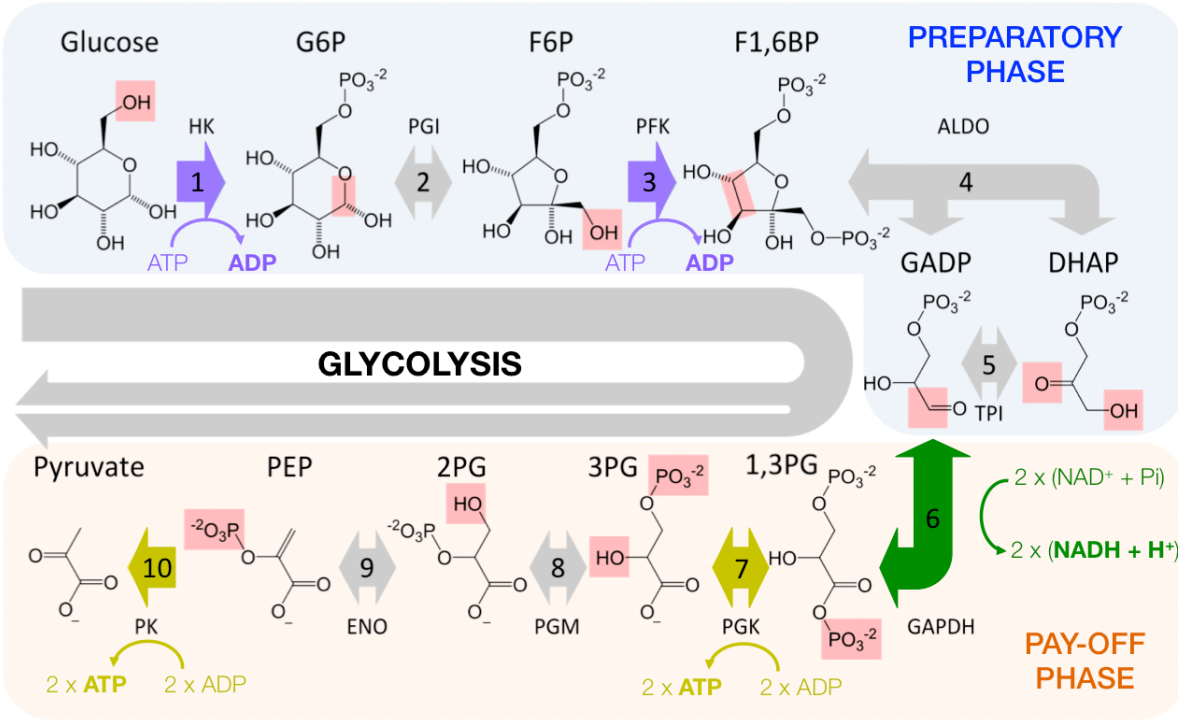


Figure 11. The metabolic pathway of glycolysis. Glycolysis converts glucose to pyruvate. Each chemical modification (red box) is performed by a different enzyme. Glycolysis can be separated in two phases: the preparatory phase and the pay-off phase. The preparatory phase has 5 reactions. ATP is consumed in the irreversible reactions number 1 and 3 (purple arrows). In the reaction number 4, F1,6BP (6 molecules of carbon) is split in 2 molecules: GADP(3 molecules of carbon) and DHAP (3 molecule of carbon). The pay-off phase has 5 reactions. NADH is synthetized in the reaction number 6 (green arrow). ATP is produced in the reactions number 7 and 10 (yellow arrows). The reaction number 10 is irreversible. Enzymes: HK, hexokinase, G6P, glucose-6-phosphatase; PGI, phosphoglucose isomerase PFK, phosphofructokinase; ALDO, aldolase; DHAP, dihydroxyacetone phosphate; GAPD, glyceralde 3-phosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENO, enolase; PK, pyruvate kinase. Metabolites: G6P, glucose-6-phosphato; F6P, fructose-6-phosphatase; F1,6BP, fructose-1,6-biphosphatase; GADP, glyceralde 3-phosphate; DHAP, dihydroxyacetone phosphate; 1,3PG, 1,3-biphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate, PEP, phosphoenolpyruvate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; NAD^+, nicotinamide adenine dinucleotide oxidized; NADH, nicotinamide adenine dinucleotide reduced. Draw modified from Thomas Shafee.

allowing for more glucose uptake. Furthermore, G6P cannot be transported out of the cell, since there are no G6P transporters on the membrane.

2. G6P is interconverted into fructose 6-phosphate (F6P) by glucose phosphate isomerase (PGI). In addition, G6P can decarboxylated to form ribose-5- phosphate in a series of reactions known as pentose-phosphate pathway.
3. Phosphorylation of F6P to fructose-1,6-biphosphate (F1,6BP) catalyzed by the allosteric enzyme phosphofructokinase (PPK) using ATP as energy source. There are three phosphofructokinase genes in humans. This reaction is irreversible.
4. The fourth reaction involves the breakdown of F1,6BP (6 carbons) into two triose sugars, glyceralde 3-phosphate (GADP) that is an aldose, and dihydroxyacetone phosphate (DHAP), which is a ketose. This reaction is catalyzed by the enzyme aldolase (ALDO).
5. Glyceralde 3-phosphate (GADP) can be interconverted to dihydroxyacetone phosphate (DHAP) by enzyme triose phosphate isomerase (TPI).

❖ The Pay-Off Phase: in this second stage, energy is produced either in the form of ATP or $NADH+H^+$, which can be later converted to ATP in the mitochondria via electron transport chain (ETS). Since glucose is converted to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This stage produces four ATP molecules and two NADH molecules and includes the last 5 reactions.

6. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) converts GADP to 1,3-biphophoglycerate (1,3BPG). GAPDH is dehydrogenated and the hydrogen released is used to reduce NAD^+, a hydrogen carrier, to give $NADH + H^+$.

7. In the seventh reaction 1,3BPG is interconverted to 3-phosphoglycerate (3PG) catalyzed by phosphoglycerate kinase (PGK) generating one ATP molecule. Two isoenzymes of PGK have been identified in mammals.
8. This reaction includes the isomerization of 3PG to 2-phosphoglycerate (2PG) catalyzed by the enzyme phosphoglycerate mutase (PGM)
9. Enolase (ENO) catalyzes the conversion of 2-PG to phosphoenolpyruvate (PEP). Three major isoforms of ENO have been identified in mammals.
10. The final reaction of the glycolysis involves the dephosphorylation of (PEP) to pyruvate (Pyr) by the enzyme pyruvate kinase (PK) generating one molecules of ATP. PK is allosterically activated by PEP and negatively regulated by ATP. This reaction is irreversible.

(Berg 2002, Pelicano 2006, Bolaños 2010, Nelson 2012)

The metabolic fate of pyruvate depends on the tissue and environmental conditions. When oxygen is present, Pyr is interconverted to acetyl-coenzyme A (acetyl-CoA) in the mitochondria, where it is fully oxidized to CO_2 through the citric acid cycle, also known as tricarboxylic acid cycle (TCA) or Krebs cycle. This process is called aerobic respiration. However, when insufficient oxygen is available or any component of the mitochondrial respiratory chain is impaired, Pyr is converted to lactate coupled with an oxidation of NADH to NAD^+ by the enzyme lactate dehydrogenase (LDH). This process is known as fermentation (Bolaños 2010, Pelicano 2006). Glycolysis and lactic acid fermentation are much increased in cancer cells even if oxygen is plentiful. This pathogenic event is called Warburg effect and may be a consequence of an adaptation to low-oxygen environments within tumors, mitochondria impairment in cancer cells, or an effect associated with cell proliferation (Vander-Heiden 2009).

Interestingly, mass spectrometry analysis in SCA1^{154Q} knock-in mice cerebellum showed a significant reduction of the glycolytic metabolite glucose-6-phosphate compared to WT mice (Perroud 2013). This study suggests that glycolysis misregulation is induced by the pathogenic SCA1^{154Q} in these mice.

1.4.3. Glucose metabolism in neurological disorders

Caloric restriction (CR) is a dietary regimen that reduces calorie intake without incurring malnutrition or a reduction in essential nutrients such as vitamins and amino acids. CR has been proved to have a strong correlation with lifespan extension and to improve health by delaying the onset of age-related diseases including neurodegenerative diseases (Walford 1987, Weindruch 1988, Mair 2008). This phenomenon is conserved across phyla as shown in a variety of studies from unicellular organisms to primates (Schulz 2007, Colman 2014).

The benefits associated with CR are mediated by multiple pathways including glycolysis (Schulz 2007, Yao 2011). Thus, to evaluate the implication of glycolysis in CR benefits, glycolysis inhibitors are often used instead of restrictive dietary regimen. 2-deoxy-D-glucose (2-DG) is a glucose analog compound, which has been used over years to inhibit glycolysis (Sols 1954, Combs 1986, Pelicano 2006). 2-DG is uptaken by glucose transporters into the cell and is phosphorylated (2-DG-P) by hexokinase in the first step of the glycolysis. 2-DG-P cannot be further metabolized by phosphate isomerase and is therefore accumulated in the cytoplasm interfering with sugar metabolism by inhibiting glycolytic enzymes (Sols 1954, Wick 1957, Chen 1992). 2-DG is widely used in anti-cancer treatments with the purpose to abolish Warburg effect and it is in clinical trial . In addition, 2-DG was also used in the context of aging studies. For example, downregulation of glycolysis using 2-DG extends life span in Caenorhabditis elegans by inducing mitochondrial respiration and increasing oxidative stress (Schulz 2007). In addition,

2DG reduces Aβ pathology in mouse model of Alzheimer's Disease (AD) by maintaining mitochondrial bioenergetic function (Yao 2011). 2-DG was also employed in other studies to reduce ATP production, revealing the upregulation of the proteasome activity under low ATP levels conditions. This suggests that 2DG can be use as a new strategy to manipulate proteasome activity by altering ATP endogenous levels (Huang 2010) (Fig 12).

Accumulation of misfolded proteins and aggregates are characteristic in numerous late-onset neurodegenerative diseases such as HD, SCA1, Parkinson's disease (PD) and AD (DiFiglia 1997, Ross 1997, Lippens 2007, Gundersen 2010). Molecular chaperones and ubiquitin–proteasome system (UPS) are essential to maintain proteins in properly folded states. This machinery start to fail in aged cells (Ben-Zvi 2009). Proteostasis networks deteriorate as a function of time and make cells more vulnerable to proteotoxic events (Douglas 2010). Based on these observations, many researchers are investigating dietary restriction, or glycolysis downregulation more specifically, as a target to delay the proteotoxic onset caused by different conformational disease proteins (Luchsinger 2002, Schulz 2007, Steinkraus 2008, Douglas 2010).

Other studies established a link between glucose metabolism and autophagy (Rabinowitz 2010). Autophagy is a mechanism for degradation and recycling of cellular components such as proteins and organelles in lysosomes (Kaushik 2010). There are three types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) (Lee, 2009). Macroautophagy is a well-established process in which double-membraned structures called autophagosomes enclose cellular material and then fuse with lysosomes where the material is degraded (Yang 2010). Microautophagy is a non-selective lysosomal degradative process where cytoplasmic cargo is invaginated into the lysosome lumen (Li 2012). In the case of CMA, proteins are targeted to

lysosomes where they are recognized by Hsc70 chaperone and then translocated into its lumen where they are degraded (Cuervo 2014).

Initially, autophagy was described as an alternative source of energy when nutrients are limited. Low nutrient condition activates autophagy through AMPK-mTORC1 signaling pathway inhibition (Rabinowitz 2010) (Fig 12). The mechanism of signal integration between glycolytic and TORC1/autophagy pathways has not been yet elucidated. A previous study suggests the participation of hexokinase II

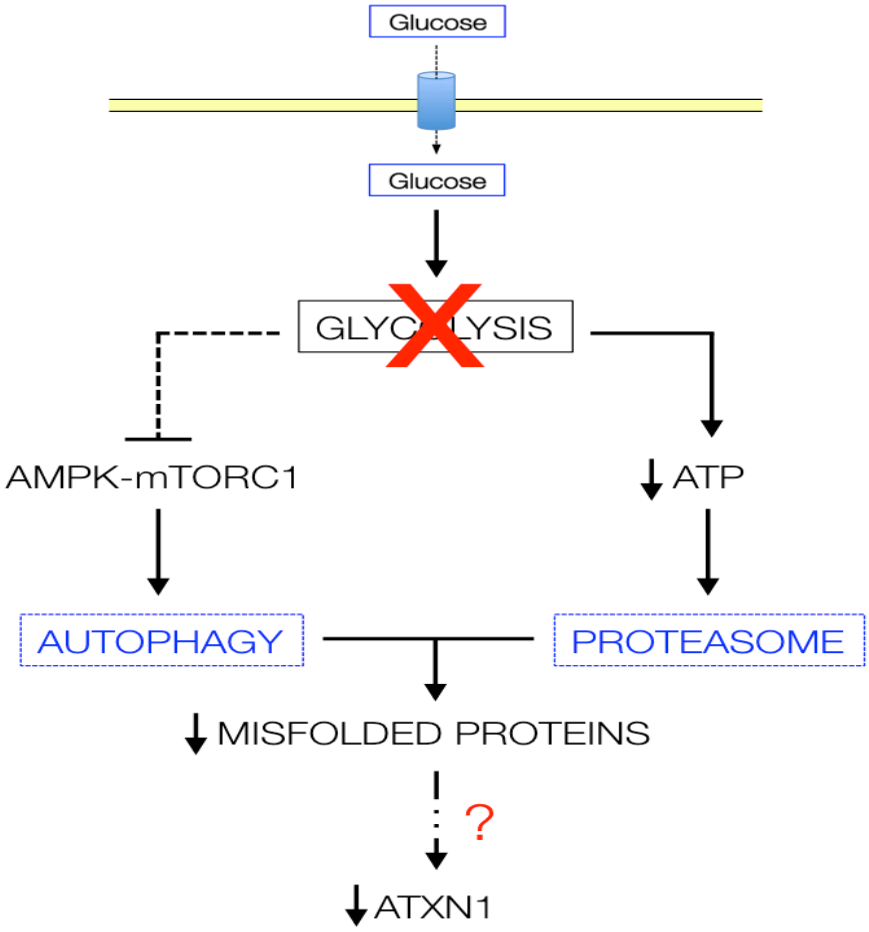


Figure 12. Downregulation of glycolysis activates autophagy and proteasome activity. Glycolysis downregulation produces an inhibition of AMPK-mTORC1 signaling pathway, thus autophagy is activated and then misfolded proteins can be degradated (Ravikumar 2004), (Rabinowitz 2010). Downregulation of glycolysis also leads a decreasing of ATP levels that trigger the activation on the proteasome, whose function is to degrade damaged proteins (Huang, 2010). Both mechanisms can potentially degrade ATXN1 mutated protein levels

(HK-II) in this process. It showed that HK-II binds to TORC1 through its TOS motif, decreasing TORC1 activity, and thereby positively regulates protective autophagy (Roberts 2014).

New findings provide an association between autophagy and neurodegenerative processes. Several studies show an increase in neurodegenerative events when autophagy is impaired in mice neuronal cells (Komatsu 2006, Hara 2006). Other studies in AD and PD detected autophagy dysfunction leading to accumulation of aberrant proteins (Nixon 2007, Pan 2008). In the case of HD, activation of autophagy reduces toxicity caused by mutated huntingtin protein in fly and mouse models by attenuating huntingtin accumulation (Ravikumar 2004). A link between neurodegenerative pathology, glucose metabolism impairment, and autophagy alteration has not been assessed.

OBJETIVES

2. OBJETIVES

A previous study revealed impairment in insulin sensitivity and insulin secretion in normoglycemic SCA1 (Lalić 2010). In addition, mass spectrometry analysis in SCA1^{154Q} knock-in mice cerebellum showed a significant reduction of the glycolytic metabolite glucose-6-phosphate compared to WT mice (Perroud 2013).

Glycolysis inhibition leads to ATP level reduction, upregulating proteasome activity (Huang, 2010). Other studies established a link between glucose metabolism and autophagy (Rabinowitz 2010).

I hypothesize that SCA1 pathogenesis impairs glucose metabolism and may modulate ATXN1 protein levels.

In order to test this, I have considered the following specific objectives:

- Study glucose uptake in SCA1 mice model using positron emission tomography – fludeoxyglucose F^{18}.
- Transcriptomic analysis in SCA1 mice and fly models to study possible alterations in glucose metabolism genes expression profiling.
- Metabolomic analysis in SCA1 *Drosophila* model to study levels of glycolytic metabolites.
- To study genetic interactions, carry out a genetic screen in *Drosophila* of all genes encoding glycolysis enzymes for potential modifier genes of SCA1. Evaluate if transcriptional changes observed in RNA sequencing are deleterious or compensatory.
- Study if the potential modifiers found can modulate ATXN1 protein levels.

MATERIAL AND METHODS

3. MATERIALS AND METHODS

3.1. *Drosophila* kinase screen

The previously characterized y,w,UAS-ATXN1^{82Q}(line-F7);GMR-GAL4 line (Fernandez-Funez 2000) were used for the screen. shRNA (Figure 13) and loss-of-function alleles were obtained from the Vienna *Drosophila* RNAi Center (http://stockcenter.vdrc.at/control/main) and the Bloomington *Drosophila* Stock Center at University of Indiana (http://flystocks.bio.indiana.edu) repositories. Animals were crossed and kept at 25 °C for the test of retinal degeneration.

For scanning electron microscopy (SEM) images, whole flies were dehydrated in ethanol, critical-point dried in a Blazers Union FL-9496 model, covered with silver and analyzed with the JEOL JSM 6100 microscope. For sections of adult fly eyes, adult heads (1 day of age) were fixed over-night in 85% ethanol, 5% acetic acid and 3.5% formaldehyde, followed by dehydration in ethanol and embedded in paraffin. Vertical sections of 10μm were made with Olympus Cut 4055, and then stained with hematoxylin. They were analyzed with a Nikon Microphot FXA microscope.

3.2. *Drosophila* motor performance tests

ATXN1^{82Q} flies were crossed with transgenic flies carrying glycolytic genes shRNA (Figure 13) or p-element loss of function mutations. They were monitored to evaluate the effect of glycolytic enzymes in the pathogenesis caused by ATXN1^{82Q} in flies. Flies were raised at 28.5 °C. Flies are transferred to vials containing new food every day. Fifteen age-matched virgin females were placed in a 35 cm clean vial and tapped down to the bottom. The number of flies that climbed up 9 cm in 15 s was recorded with infrared detector. We repeated this ten consecutive times

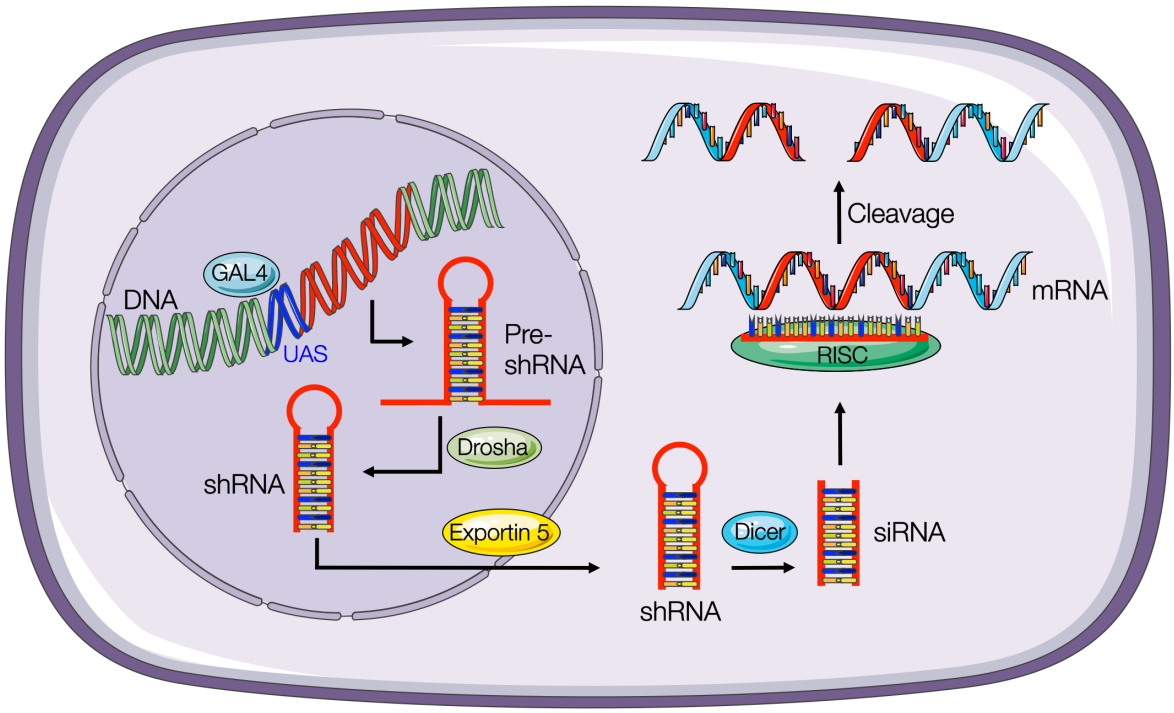


Figure 13. Mechanism of small hairpin RNA (shRNA). shRNA is transcribed in the nucleus by polymerase. The product is processed by Drosha and exported from the nucleus by Exportin 5. This product is then processed by Dicer and loaded into the RNA-induced silencing complex (RISC). The sense strand (passenger) is degraded and the antisense strand (guide) directs RISC to messenger RNA (mRNA) that has a complementary sequence. In the case of perfect complementarity, RISC cleaves the mRNA. In the case of imperfect complementarity, RISC represses translation of the mRNA. In both of these cases, shRNA leads to target gene silencing. I use flies that carry shRNA with UAS as a promoter, thus shRNA will be transcribed only in presence of GAL4.

and the average of the ten observations was plotted for each day as shown in the chart. Two replicates were tested in parallel for each genotype. ATXN1(82Q) was expressed in the nervous system using nrv2-GAL4 (y,w,UAS-ATXN1(82Q)(F7) nrv2-GAL4).

3.3. Preparation of *Drosophila* protein lysates and immunoblot

For the Western Blot I used eight fly heads per genotype at 25.5°C. Protein lysates were prepared in NuPAGE LDS sample buffer (Invitrogen), and then run in NuPAGE Novex 4-12% Bis-Tris Protein Gels, 1.0 mm, 10 well. The proteins are

transferred to a nitrocellulose membrane for 2 hours at 200 V. Primary antibodies used were 11NQ anti-ATXN1 rabbit (Neuromab) 1:2000 and anti-laminC mouse to detect the loading control (Developmental Studies Hybridoma Bank) 1:2000.

3.4 Metabolomics analysis in flies. NMR

Female mice aged 9 weeks were anesthetized using Isoflurane before cervical dislocation. Cerebellum and brain stem were extracted from the whole brain, dissected into ≈5mm slices and placed in individual 1.5 ml eppendorf tubes. Tissue samples were snap frozen in liquid nitrogen, weighed and stored at -80 °C until the analysis.

Tissues were smashed briefly in eppendorfs tubes whilst frozen with single-used plastic pellets. 100 μl of ice-cold 1XPBS were added to the smashed tissue and further homogenized using a motorized hand pestle. A further 900 μl of ice-cold 1XPBS were added to the homogenized tissue and mixed well. Samples were placed on dry ice and sent to the NMR facility at University of Houston for analysis.

The aqueous extracts are obtained in 500 μL of reconstitution buffer with lmM TSP as an internal standard. The organic extracts are reconstituted in 500 μL of CDCl3 with 0.03% TMS as an internal standard. The NMR acquisition then involves locking to the solvent and shimming to achieve optimal line-shape. Specific pulse-sequences are applied depending on the experimental hypothesis. Typically, ID ^{1}H NMR includes zgpr, noesyprld (for single solvent suppression) and lclpnf2 (for double-solvent suppression. Typical 2D NMR comprises COSY and TOCSY sequences. Hetero nuclear experiments include 1H-13C HSQC and HMBC sequences. The obtained data are analyzed and the set of metabolites is determined. In dubious cases, the specific metabolites can be validated by spike-in experiments.

3.5. Network analysis of modifiers

Network analysis was performed using Ingenuity Pathway Analysis (IPA). IPA Downstream Effects Analysis predicts the expected increase or decrease of gene functions, based on the experimental data. Downstream Effects Analysis compiles information from the literature into the Ingenuity Ingenuity Knowledge Base. The analysis examines genes in the data that are known to perform certain functions, compares the genes' direction of change to expectations derived from the literature, then issues a prediction for each function based on the direction of change. The direction of change is the gene expression in the experimental samples relative to a control. If the direction of change is:

- Consistent with the literature across most genes, IPA predicts that the function will increase in the experimental sample.
- Mostly inconsistent with the literature, IPA predicts that the function will decrease in the experimental sample.
- Not clear (there is no clear pattern related to the literature), IPA does not make a prediction.

IPA uses the z-score algorithm to make predictions. The z-score algorithm is designed to reduce the chance that random data will generate significant predictions. The score is the negative log of the p-value derived from the Fisher's Exact test.

3.6. Transcriptomics analysis. RNA-sequencing

The Institutional Animal Care and Use Committee approved all animal use protocols. ATXN1[82Q] (Burright 1995) mice and were housed and managed by Research Animal Resources under SPF conditions in an AAALAC-approved facility. Whole cerebellar RNA from three biological replicates for each genotype was isolated using TRIzol Reagent (Life Technologies) and then purification using

RNAeasy Kit following the manufacturer's procedure (Qiagen). Purified RNA was tested for quality control, including quantification using fluorimetry (RiboGreen assay, Life Technologies) and RNA integrity assessed with capillary electrophoresis (Agilent BioAnalyzer 2100, Algilent Technologies, Inc.) generating an RNA integrity number (RIN). All submitted samples had greater than 1ug total mass and RINs greater than 8. Library creation was completed using oligo-dT purification of polyadenylated RNA, which was reverse transcribed to create cDNA. The cDNA was fragmented, blunt - ended, and ligated to barcoded adaptors. The library was size selected to 320bp +/- 5% to produce average inserts of approximately 200bp, and size distribution validated using capillary electrophoresis and quantified using fluorimetry (PicoGreen, Life Technologies) and Q-PCR. The libraries were then normalized, pooled and sequenced. Samples were sequenced on an Illumina GAIIX using a 76nt paired-end read strategy.

Initial read quality was determined using FastQC to determine the following: basic statistics, per base sequence quality, per sequence quality scores, per base sequence content, per base GC content, per sequence GC content, per base N content, sequence length distribution, sequence duplication levels, overrepresented sequences, kmer content. Using the FastQC results, reads were trimmed to an acceptable length. Reads were processed to remove contaminating adapter sequences. All paired-end reads were correctly synchronized.

Reads were aligned to the mouse reference genome (Illumina igenomes mm9) with Tophat by using mostly default parameters, except a Std. Dev for Distance between Mate Pairs of 60 and using a gene annotation model only looking for supplied junctions. Differential gene expression was determined with Cuffdiff using default parameters. Splicing analysis was completed using GSNAP and DEXseq by using most default parameters. Genes/introns with a $q \leq 0.05$ were considered significant.

3.7. MRI

MR images were obtained using a Bruker Avance BioSpec Spectrometer with a 21cm horizontal bore (Bruker BioSpin, Billerica, MA) and a 75mm resonator. A rapid acquisition with relaxation enhancement (RARE) scan with the following scan parameters: TE=11ms, TR3817.9ms, RARE factor=8, # of averages=2, Total scan time=4m4s344ms was used to obtain images of the entire brain (Fig 14).

3.8. PET-FDG

All PET and CT images were obtained using an undocked Inveon scanner (Siemens AG, Knoxville, TN). Mice were fasted for 4 hours prior to injection of 18F-FDG. Each animal received 340 μCi of radioisotope (Cyclotope, Houston, TX) via IV tail vein injections while awake. Blood glucose levels were also assessed prior to infusion with 18F-FDG. One-hour post-injection of radioisotope, mice were anesthetized with 2% isoflurane in oxygen and placed on the imaging bed. Breathing and body temperature were monitored and maintained using a BioVet animal monitoring system (BioVet, Newark, NJ). A CT scan was acquired containing 220 projections covering 220°, with a source-to-detector distance of 312.91 mm and source-to-center of rotation (COR) distance of 183.92 mm. Each projection was 650 ms with x-ray tube voltage and current respectively at 80 kVp and 500 μA. The PET scan was reconstructed using OSEM3D reconstruction and registered to the CT scan. Mice were imaged for 5 minutes (CT) and 30 minutes (PET) starting one hour post-injection of the radioisotope (Fig 14).

3.9. γ - Counting

γ -Counter measurements were used to corroborate the 18F-FDG measurements using a γ -Counter (Wizard 2 2480; PerkinElmer, Waltham, MA). Mouse brain tissue was harvested after PET-FDG imaging immediately post-imaging. The 18F-FDG radioactivity was counted specifically within the cortex, cerebellum and blood was

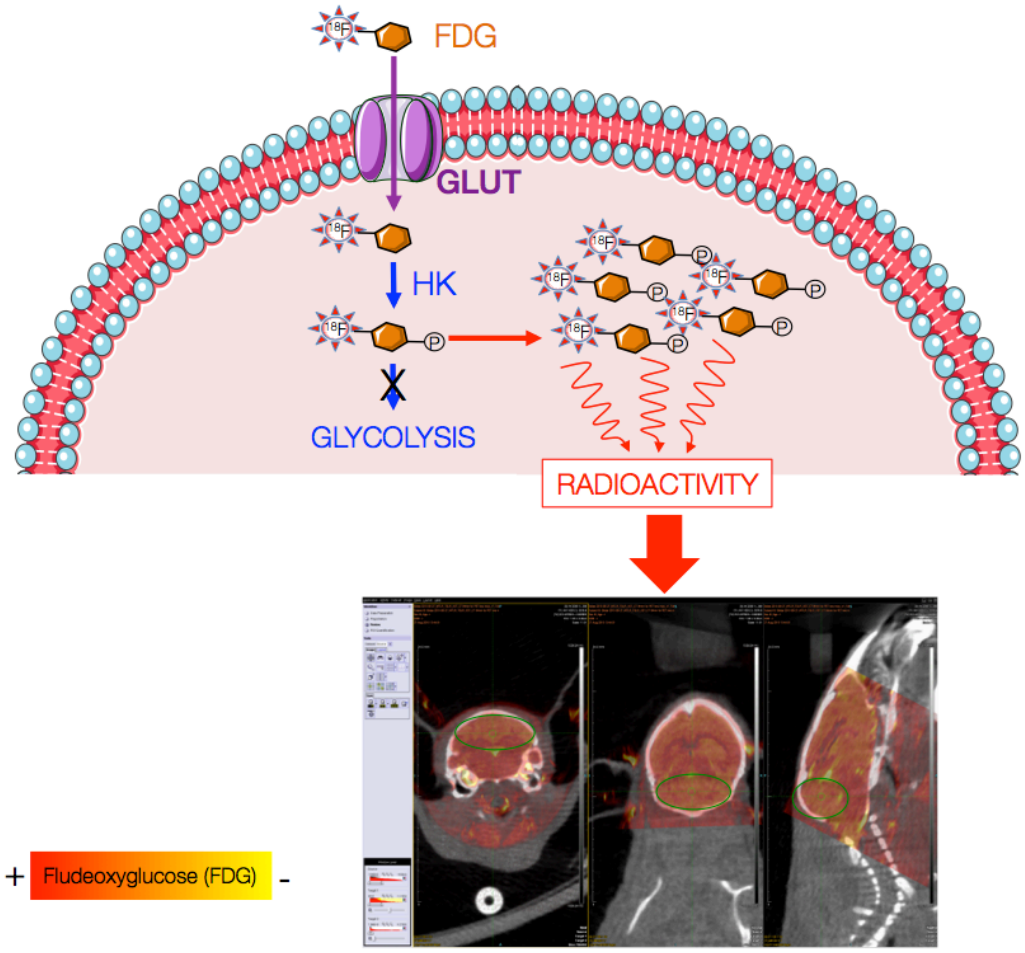


Figure 14. Positron emission tomography (PET) – magnetic resonance imaging (MRI). Fludeoxyglucose (FDG), a glucose analog with the positron-emitting radioactive isotope fluorine-18 (^{18}F) is transported into cells through glucose transporters and it is converted by the enzyme hexokinase to FDG phosphorylated (FDG-P). FDG-P cannot undergo further in glycolysis and tends to accumulate in the cytoplasm. Since positron emission tomography (PET) only detects radioactivity emitted by ^{18}F, we cannot distinguish the different brain regions, mice were also imaged using MRI with the objective to identify the cerebellum portion (delimited by a green line) in the image obtained by PET. Finally, images obtained by PET and MRI were overlapped to quantify radioactivity emitted by the cerebellum area.

measured and corrected for radionucleotide decay from time of injection, and also normalized to injected dose and tissue weight. Data was reported as % injection dose per gram.

3.10. PET Image Analysis

MRI brain scans were registered manually with the PET/CT images using Inveon Research Workplace (Siemens AG, Knoxville, TN). Once, this was done, the whole brain and cerebellum of each mouse was segmented into distinct regions of interests (ROI). These ROIs were then used to measure the uptake of radioisotope into each of the segmented parts of the brain.

3.11. Statistical analysis

Western Blot: protein samples were run three independent times, and were normalized using LamC as loading control. P-value was calculated with Student's t-test (tails: 2, type: 1).

Motor performance tests: two replicates were tested in parallel for each genotype and they were repeated twice. Statistical analysis was performed with Prism (GraphPad, La Jolla, CA). Each time point was analyzed using two-way ANOVA followed by two-way ANOVA followed by Sidak's multiple comparison tests.

PET and γ – Counting: Voxel intensity is reported as the mean ± standard deviation. Statistical analysis was performed with Prism (GraphPad, La Jolla, CA). T-test was used to compare multiple means with statistical significance set at $p < 0.05$.

RESULTS

4. RESULTS

4.1. Decreased glucose import into the cerebellum of ATXN1^{82Q} transgenic mice

Glucose is absorbed into the bloodstream during digestion, travels along the circulatory system and crosses the brain blood barrier in order to supply energy to central nervous system cells. Glucose is imported into cells through transporters that are activated via insulin signaling and are localized in the plasma membrane,. A previous study revealed that in SCA1 patients, insulin sensitivity and insulin secretion are impaired (Lalić 2010). In addition, SCA1 model of ATXN1^{154Q} knock-in mice showed a decrease in glucose-6 phosphate glycolysis metabolite (Perroud 2013). I hypothesize that the expression of mutant ATXN1 causes an impairment of glucose import.

The B05 transgenic mouse model expresses mutant human ATXN1 with 82 repeats in the cerebellum using the cerebellum Pcp2 promoter (Burright 1995). Thus, in principle, the cerebellum is the only brain region that is affected in these mice. To measure the level of glucose import by cerebellar cells I carried out positron emission tomography (PET) and magnetic resonance imaging (MRI) analysis in 14 week-old B05 mice (Fig 15). These mice were injected with fludeoxyglucose (FDG), a glucose analog where the hydroxyl group at the 2' position in the glucose molecule is substituted with the positron-emitting radioactive isotope fluorine-18 (^{18}F). FDG is transported into cells through glucose transporters and is converted to phosphorylated FDG (FDG-P) by the enzyme hexokinase. FDG-P cannot proceed further in glycolysis and therefore accumulates in the cytoplasm. Since PET only detects radioactivity emitted by ^{18}F, I cannot distinguish the different brain regions. Therefore, B05 mice were also imaged using MRI, which allow the identification of the cerebellum portion in the image obtained by PET (Fig 15). I tested the blood glucose levels before the PET imaging. All animals were normoglycemic and

showed similar glucose levels to WT mice. Analysis of the images obtained by PET revealed glucose import impairment in SCA1 transgenic mice, which suggest lower glucose level in cerebellar cells (Fig 15).

To confirm this result, I carried out gamma counting analysis (Fig 15). I extracted the cerebellum and measured the radioactive emission of the whole organ using a gamma counter. The total radioactivity was lower in the cerebellum of SCA1 transgenic animals than that in WT, confirming the results from the PET analysis (Fig 15).

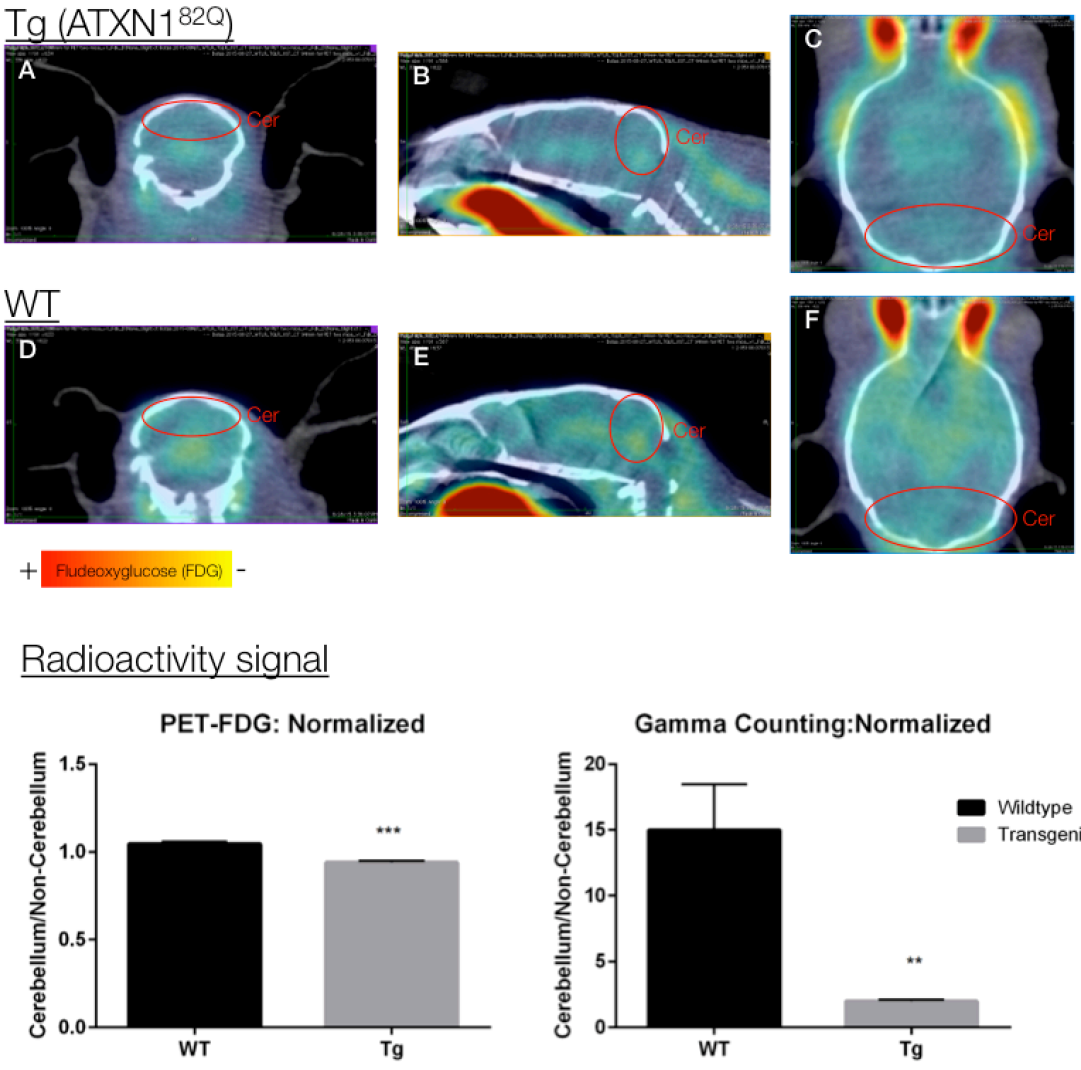


Figure 15. Positron emission tomography (PET) - magnetic resonance imaging (MRI) and gamma counting reveal glucose import impairment in cerebellum ATXN1^{82Q} transgenic mice using fludeoxyglucose F^{18} as a radioactive isotope. A-F show images obtained by (PET) overlapped with images obtained by (MRI) in axial (A and D), sagittal (B and E) and coronal (C and F) sections. A-C correspond to ATXN1^{82Q} transgenic (Tg) mice and D-F correspond to wild type (WT) mice. Cerebellum area is delimitated using a red line. I observe a decrease in radioactivity intensity signal in cerebellum of Tg compared WT mice. G graph shows radioactivity signal quantification normalized (whole brain – cerebellum) from PET-MRI revealing a decrease in fludeoxyglucose F^{18} (FDG) signal in Tg compared WT mice cerebellum. H graph shows radioactivity signal quantification normalized (whole brain – cerebellum) from gamma counting and confirms a significantly lower level of FDG in the cerebellum of the Tg compared WT. (N=5, normalized: T-test, statistic: two-way ANOVA with multiple comparisons, **p<0.05, ***p<0.005)

4.2. Expression of glucose metabolism genes is altered in SCA1 mice cerebellum and SCA1 flies nervous system.

Insulin activates insulin receptors, which leads to the activation of glucose transporters that allow the entry of glucose into cells where glucose is metabolized. Since MRI-PET studies suggested the impairment of glucose uptake, I investigated the possibility of altered expression of genes involved in glucose metabolism. RNA sequencing (RNAseq) was performed to analyze the transcriptomes of ATXN1^{82Q} transgenic mice (B05 mice) (at two different time points), ATXN1^{54Q} knock-in mice, and ATXN1^{82Q} transgenic flies.

4.2.1. RNAseq in B05 ATXN1^{82Q} transgenic mice

Total RNA was extracted from B05 and WT/FVB cerebellums. Since the disease shows a progression, I used two different time points to gain insight into the course of the disorder: five weeks of age, when animals show a mild phenotype; and twelve weeks of age, when the phenotype is much stronger.

The RNAseq data of five-week old B05 mice showed an altered expression level of 336 transcripts, of which 33 (9.8% of total altered genes) are genes that participate in glucose metabolism (Fig 16). These data suggest an alteration in the expression pattern of glucose metabolism genes. Interestingly, Scl2a1, a glucose transporter showed a downregulation compared with the wild-type mice, which suggests an impairment in glucose uptake (Fig 17). I analyzed all transcripts that show an alteration using the informatics platform Ingenuity Pathways Analysis (IPA). IPA predicts the effect of gene expression changes in the dataset of biological processes. In this case I analyzed glucose uptake from the RNAseq data. This analysis did not predict any inhibition or activation of glucose uptake in B05 mice five-weeks old (Fig 16). However, it is important to note that IPA gives the same consideration to each gene that participates in a particular process. Thus, I should

perform further analysis for a more accurate result. For example, in this case Slc2a1 glucose transporter gene transcript is downregulated, therefore it suggests a likely downregulation in glucose uptake process independent of the result obtained using IPA analysis.

The RNAseq of twelve-week-old B05 mice showed altered expression in 1493 genes, which is 4.5 times more than the number of gene expression changes in five-week old mice (Fig 16). This result is consistent with the progression of the disease severity. I found 133 (8.9% of total altered genes expression) altered gene expression level in glucose metabolism genes compared to WT mice (Fig 16). Furthermore, I found Slc2a13 glucose transporter, insulin receptor (InsR), and some glycolytic enzymes (Hk2, AldoC, Gapdh, Eno1b and Pfkfb3 genes) were all downregulated, suggesting the decrease of glucose uptake and glycolytic flux (Fig 17). I also performed IPA analysis and the results confirmed the glucose uptake inhibition (Fig 16).

4.2.2. RNAseq in ATXN1^{154Q} knock-in mice

Total RNA was extracted from knockin and wild-type cerebellums in eight-week old animals. I found 421 transcripts altered in KI mice compared with WT. Among those genes, I found 45 (10.5% of total altered genes) that are involved in glucose metabolism (Fig 16). I found that the expression levels of both Slc5a1, which is a sodium/glucose cotransporter, and ALDOC, which is a glycolytic enzyme were downregulated (Fig 17). These data suggest glucose metabolism dysregulation. I also performed IPA analysis and the result suggests that glucose uptake in the cerebellar cells in these animals was inhibited (Fig 16). This result agrees with data from the previous transcriptome analysis of B05 transgenic mice.

4.2.3. RNAseq in ATXN1^{82Q} transgenic flies

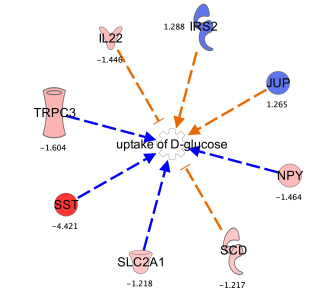
Expression profiling in both transgenic and knock-in mice suggests a misregulation of glucose metabolism genes. To test if this phenomenon is conserved across phyla I examined the transcriptome of ATXN1^{82Q} transgenic flies.

I used a pan-neuronal driver to express ATXN1^{82Q} in the *Drosophila* nervous system and I extracted total RNA from heads of 1-day-old flies. I found 359 *Drosophila* transcripts genes altered in transgenic flies compared with wild-type flies, which correspond to 566 human homolog genes (Fig 16). From these transcripts, 53 (9.4% of total altered genes) genes are involved in glucose metabolism (Fig 16). I found that expression of pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase 1 (PCK1) were upregulated. Interestingly both these enzymes play important roles in gluconeogenesis, to convert pyruvate into phosphoenolpyruvate (Fig 17). Thus, these data suggest misregulation of gluconeogenesis in SCA1 flies. IPA predicted inhibition of glucose uptake in neurons, which agrees with our finding in SCA1 mice (Fig 16).

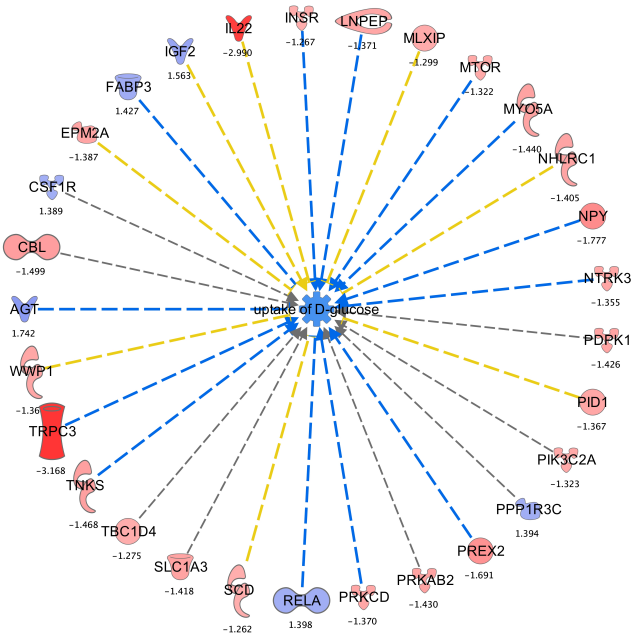
In summary, transcriptome analysis of SCA1 mice (transgenic and knock-in) and flies support the idea of impairment of glucose uptake and abnormal glucose metabolism during SCA1 pathogenesis.

	MICE				FLIES
	TG [hATXN^{82Q}] / WT		KI [mATXN^{154Q}] / WT		TG [hATXN1^{82Q}] / WT
Age	5 weeks	12 weeks	8 weeks		1 day
Total sequenced genes	23997	23997	23420		13991
Total transcripts altered	336	1493	421		359 fly genes --> 566 human homolog
	Up: 81 Down: 254	Up: 324 Down: 1169	Up: 171 Down: 250		Up: 448 Down: 188
Genes involved in glucose metabolism	33	133	45		53 human homolog
	Up: 11 Down: 22	Up: 35 Down: 98	Up: 17 Down: 28		Up: 38 Down: 15
Genes involved in glucose uptake	12	50	14		27
	Up: 2 Down: 10	Up: 13 Down: 37	Up: 6 Down: 8		Up: 19 Down: 8
	IPA prediction	IPA prediction	IPA prediction		IPA prediction
	-----	Inhibition (-1.10)	Inhibition (-1.00)		Inhibition (-1.44)

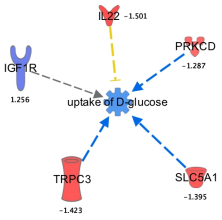
A. MICE TG [ATXN1^{82Q}] – 5 weeks



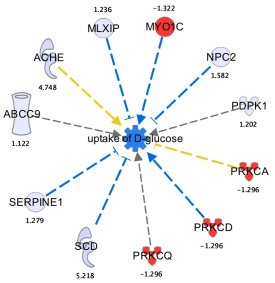
B. MICE TG [ATXN1^{82Q}] – 12 weeks



C. MICE KI [ATXN1^{154Q}] – 8 weeks



D. FLY TG [ATXN1^{82Q}] – 1 day



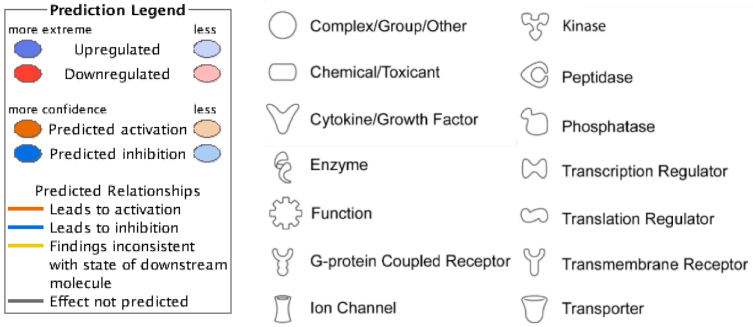


Figure 16. RNAseq reveals an impairment of glucose uptake in neuronal cells in both SCA1 mice (transgenic and knock-in) and SCA1 flies. The table shows RNAseq results summary, number of transcripts altered in each model for carbohydrates metabolism and glucose uptake processes, and the Ingenuity Pathways Analysis (IPA) predicted scores of glucose uptake in cells. (A), there was no inhibition or activation of glucose intake in five-week old B05 transgenic mice; (B), (C) and (D), inhibition of glucose uptake was observed in twelve-week old B05 transgenic mice (B), eight-week old knock-in mice (C) and flies with one-day of age (D). IPA predicted scores are summarized in the table as highlighted. Driver: elav:GAL4. The negative log of the p-value was derived from the Fisher's Exact test.

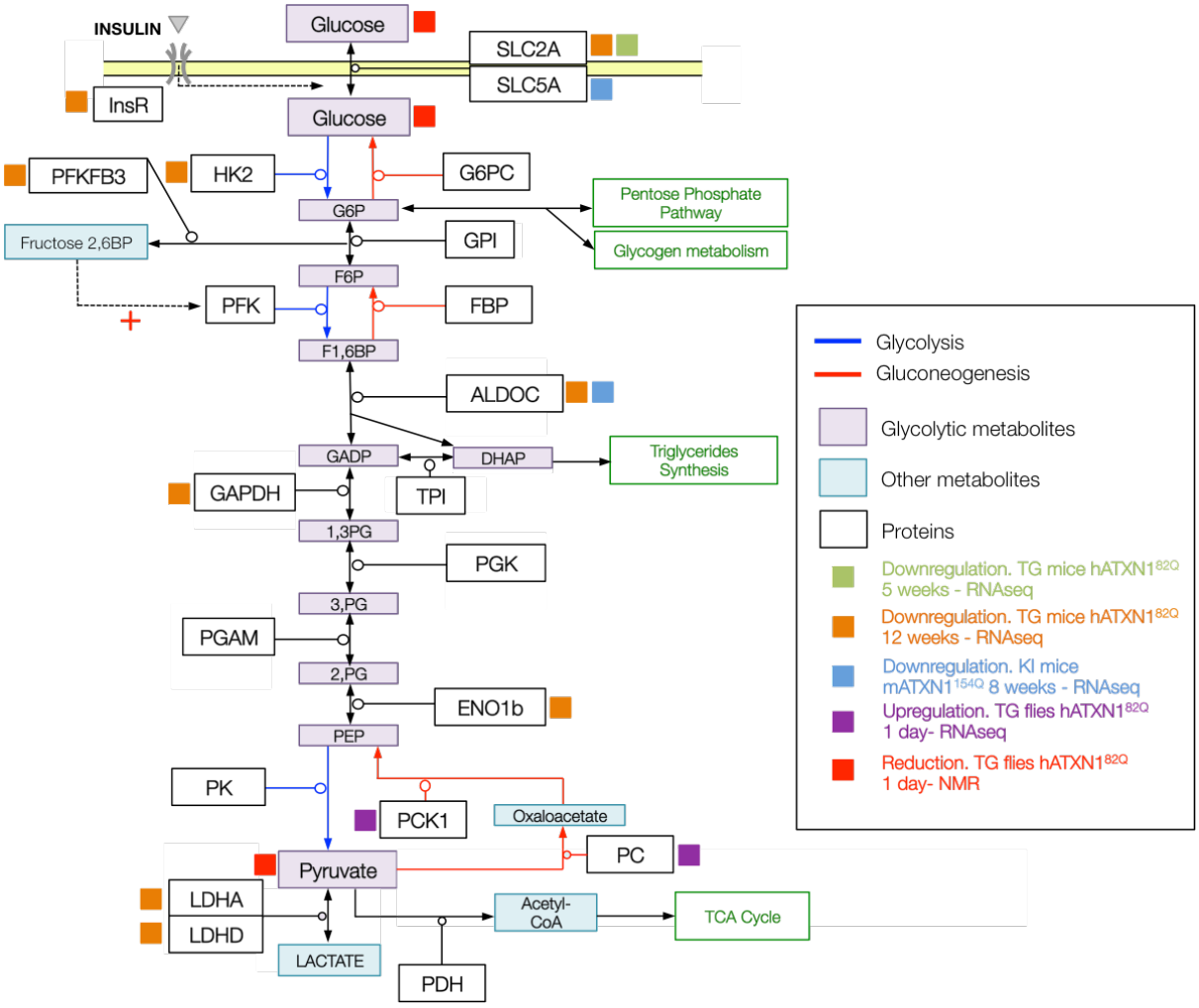


Figure 17. Glucose metabolism dysregulation in SCA1 mice (transgenic and knock-in) and fly models revealed by RNAseq and NMR. Data obtained by RNAseq in SCA1 mice (transgenic and knock-in) show a downregulation in genes that participate in glucose uptake and glycolysis. RNAseq data of SCA1 transgenic flies showed an upregulation in enzymes that participate in gluconeogenesis. NMR analysis show reduced levels in glucose and pyruvate in SCA1 flies. G6P: glucose-6-phosphate, F6P: fructose-6-phosphate, F1,6P: fructose-1,6-biphosphate, GADP: glyceraldehyde 3-phosphate, DHAP: dihydroxyacetone phosphate, 1,3PG: 1,3-bisphosphoglycerate, 3PG: 3-phosphoglycerate, 2PG: 2- phosphoglycerate, PEP: phosphoenolpyruvate, SLC2A: Solute carrier family 2, facilitated glucose transporter, SLC5A: sodium-dependent glucose cotransporter, InsR: insulin receptor, PFKFB3: fructose-2,6-biphosphatase 3, HK2: hexokinase 2, G6PC: glucose-6-phosphatase, GPI: glucose-6-phosphate isomerase, PFK: phosphofructokinase, FBP: fructose-1,6-bisphosphatase, ALDOC: aldolase C, TPI: triosephosphate isomerase, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, PGAM: phosphoglycerate mutase, ENO1b: enolase 1b, PK: pyruvate kinase, LDHA/B: lactate dehydrogenase A/B, PDH: pyruvate dehydrogenase, PC: pyruvate carboxylase, PCK: phosphoenolpyruvate carboxykinase 1, TCA: tricarboxylic acid cycle. Figure shows human homologs.

4.3. Nuclear magnetic resonance spectroscopy reveals decreased levels of glucose and pyruvate in the nervous system of SCA1 *Drosophila* model

In agreement with expression profiling studies, PET analysis showed impairment in glucose uptake in transgenic B05 and ATXN1^{154Q} knock-in mice, as well as ATXN1^{82Q} flies. Moreover, a previous study showed a G6P reduction in ATXN1^{154Q} knock-in mice revealed by mass spectrometry (Perroud 2013). Together these results suggest an alteration in glycolytic metabolites during SCA1 pathogenesis. To test this hypothesis, I performed metabolomic profiling analysis.

I carried out nuclear magnetic resonance (NMR) in transgenic files expressing ATXN1^{82Q} in the nervous system using elav:GAL4 pan-neuronal driver. NMR allows us to determine the abundance of certain glycolytic metabolites, based on the absorbance and re-emittion of molecules in a magnetic field. The peaks of

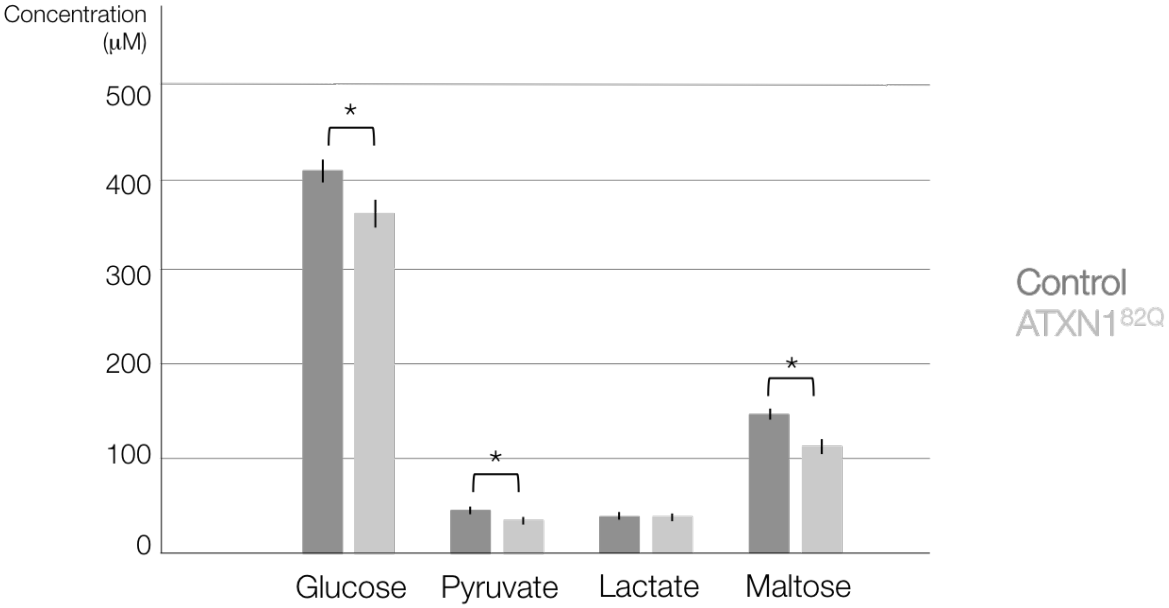


Figure 18. Reduction of glucose, pyruvate and maltose levels in ATXN1^{82Q} flies by NMR analysis . Metabolomic characterization indicates the impairment of glucose and pyruvate (glycolysis), and maltose (tricarboxylic acid cycle) in flies that express ATXN1^{82Q} in the nervous system. Driver: elav:GAL4. No change was observed in lactate level. The estimated metabolites concentrations were quantified using Chenomx. *p<0.05.

nuclear magnetic resonance spectra, correspond to different metabolites. I found a decrease in glucose, pyruvate and maltose levels, suggesting impairment in sugar metabolism. I did not identify peaks that correspond to glycolysis intermediate metabolites. No difference was found in lactate levels (Fig 17, Fig 18).

4.4. Suppression of neurodegeneration in the retina of SCA1 *Drosophila* model by reducing the activity of glycolytic genes

I found glucose metabolism impairment using different approaches in SCA1 mice and SCA1 flies. In addition, I reported a misregulation in glycolytic genes in SCA1 mice and reduction of glycolytic metabolites in SCA1 flies, suggesting impairment in glycolysis. The glycolysis pathway is the first process to metabolize glucose in cells after glucose import and it is a fundamental process in glucose metabolism. To evaluate whether the observed changes are due to pathogenic or compensatory events I assessed genetics interaction studies using our well-characterized SCA1 *Drosophila* model. Expression of ATXN1^{82Q} transgene in the fly eyes (using gmr-GAL4) triggers retinal degeneration (Fig 19). To test if glycolytic pathway modulates mutant ATXN1-induced pathogenesis, I screened flies with knock-down of each glycolic gene in SCA1 flies. The retina structure was examined at 25ºC to evaluate if reduction of the activity of glycolytic genes modifes the eye phenotype caused by ATXN1^{82Q} expression.

For this purpose, I used existing mutations (shRNA and P-element loss of function) in *Drosophila* genes encoding glycolytic enzymes and crossed them with flies expressing the *ATXN1*^{82Q} transgene. I found that retinal degeneration induced by ATXN1^{82Q} was suppressed by knock-down of all the glycolytic genes tested. (Fig 17, Fig 19). Thus, downregulation of glycolysis improves SCA1 induced retina degeneration.

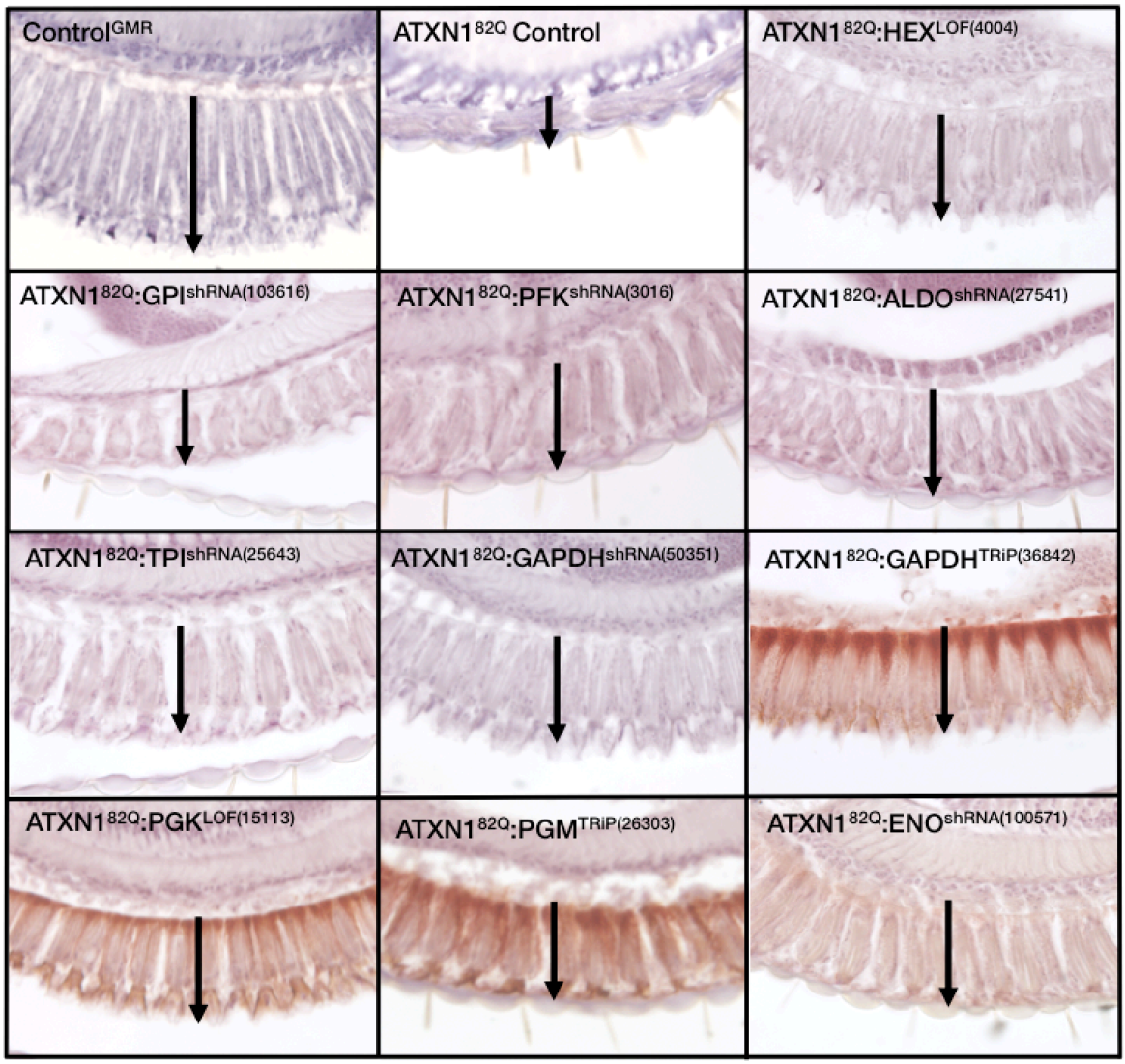


Figure 19. Knock-down glycolysis genes suppressed SCA1 induced retina degeneration and improved photoreceptor integrity. Images of paraffin sections of the retina. HEX: hexokinase, GPI: glucose-6-phosphate isomerase, PFK: phosphofructokinase, ALDO: aldolase, TPI: triosephosphate isomerase, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, PGM: phosphoglycerate mutase, ENO: enolase. shRNA: small hairpin RNA. LOF: loss of function alleles. TRiP: RNA interference. Driver: gmr:GAL4. Arrows indicate retina width.

4.5. Suppression of SCA1 behavioral deficits by knock-down of glycolytic genes in *Drosophila*

Downregulation of glycolytic genes reduced the toxicity caused by ATXN1^{82Q} expression in the fly retina. To validate these data I performed behavioral tests in flies. I expressed ATXN1^{82Q} in the nervous system using the nrv2–GAL4 pan-neuronal driver. These flies show a reproducible and quantifiable motor performance impairment measured by percentage of ATXN1^{82Q} flies able to climb into plastic tubes compared with control flies carrying nrv2–GAL4 without the SCA1^{82Q} transgene. I crossed transgenic flies carrying glycolytic genes loss of function mutations with ATXN1^{82Q} flies. They were monitored to evaluate the effect of reduced glycolytic gene activity in motor impairments caused by ATXN1^{82Q}.

I found 7 mutations (RNAi and P-element loss of function mutations) corresponding to 7 glycolytic genes (GPI, PFK, PGK, ALDO, TPI, GAPDH and PYK) that suppress ATXN1^{82Q} flies behavioral deficits (Fig 17, Fig 20). These results confirm the data obtained in ATXN1^{82Q} using the retinal neurodegeneration phenotype and support the idea that downregulation of glycolysis leads decreased ATXN1^{82Q} toxicity in the SCA1 *Drosophila* model.

In a few specific cases; ie., four RNAi constructs corresponding to glycolytic genes PGAM, GAPDH, PFK and ALDO, I found enhancement of the motor performance phenotype. For each of these four genes, additional alleles where tested in both behavioral and eye phenotype assay, and multiple alleles were identified that suppress ATXN1^{82Q} toxicity in both assays (Fig 19, Fig 20). These apparently contradictory results could arise from the different temperature used in each assay (25.5^{o}C for eye and 28.5^{o}C for motor-performance). Since UAS-GAL4 system is temperature sensible, RNAi expression is higher at temperatures closer to 30^{o}C. Thus, an increase of the gene knock-down by these RNAi constructs may cause toxicity by excessive loss of function of the corresponding genes.

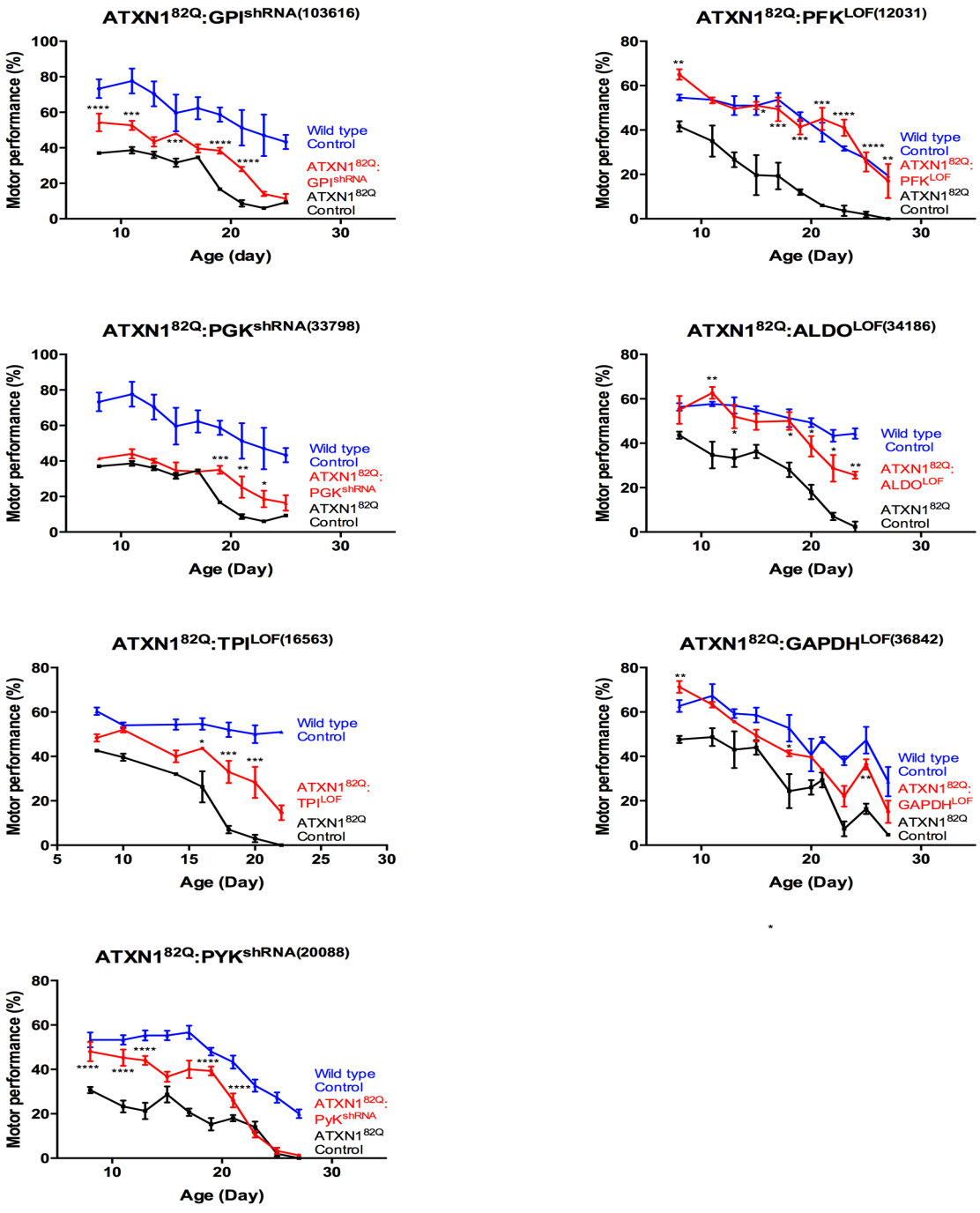


Figure 20. Knock-down of genes involved in glycolysis suppresses behavioral deficits in SCA1 *Drosophila* model. Decreased levels in the fly homologs of GPI, PFK, PGK, ALDO, TPI, GAPDH and PYK suppress ATXN1^{82Q}-induced motor impairments. GPI: glucose-6-phosphate isomerase, PFK: phosphofructokinase, PGK: phosphoglycerate kinase, ALDOC aldolase C, TPI: triosephosphate isomerase, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, PYK: pyruvate kinase. shRNA: small hairpin RNA. LOF: loss of function alleles. Driver: nrv2:GAL4. Graph lines correspond to linear regressions from 2 replicates. Statistics: experimental compared to ATXN1[82Q] control, two-way ANOVA followed by multiple comparison tests *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0005.

4.6. Decrease of ATXN1 protein levels following knock-down of the glycolytic genes in *Drosophila*

Our data indicate that genetic inhibition of the glycolysis pathway suppresses ATXN1^{82Q}–induced retinal degeneration and behavioral deficits in flies. To gain insight into the mechanism by which ATXN1^{82Q} toxicity is suppressed I investigated the possibility that reducing the activity of glycolysis genes modulates ATXN1^{82Q} protein levels.

I found that reducing levels of the *Drosophila* homologs of HEX, PGI, PFK, ALD, PGK, and GAPDH led to a decrease in ATXN1^{82Q} protein levels (Fig 17, Fig 21). I investigated the possibility that these glycolysis genes exert their effects by modulating the UAS-GAL4 system. However, as shown in Fig 22, PFK knock-down does not impact expression of a GFP reporter from the UAS-GAL4 system.

In contrast to the Western blots results described above, I found that loss of function of one specific allele (TPI^{LOF16563}) of the *Drosophila* homolog of TPI increases ATXN1^{82Q} levels (Fig 23). However, this allele worked as a suppressor in eye leading an increment in retinal thickness (Fig 23)., which suggests that ATXN1^{82Q} levels may increase because of the increment in the size of the rescued retina. This possibility will be investigated using a loading control responsive to amount of retinal tissue.

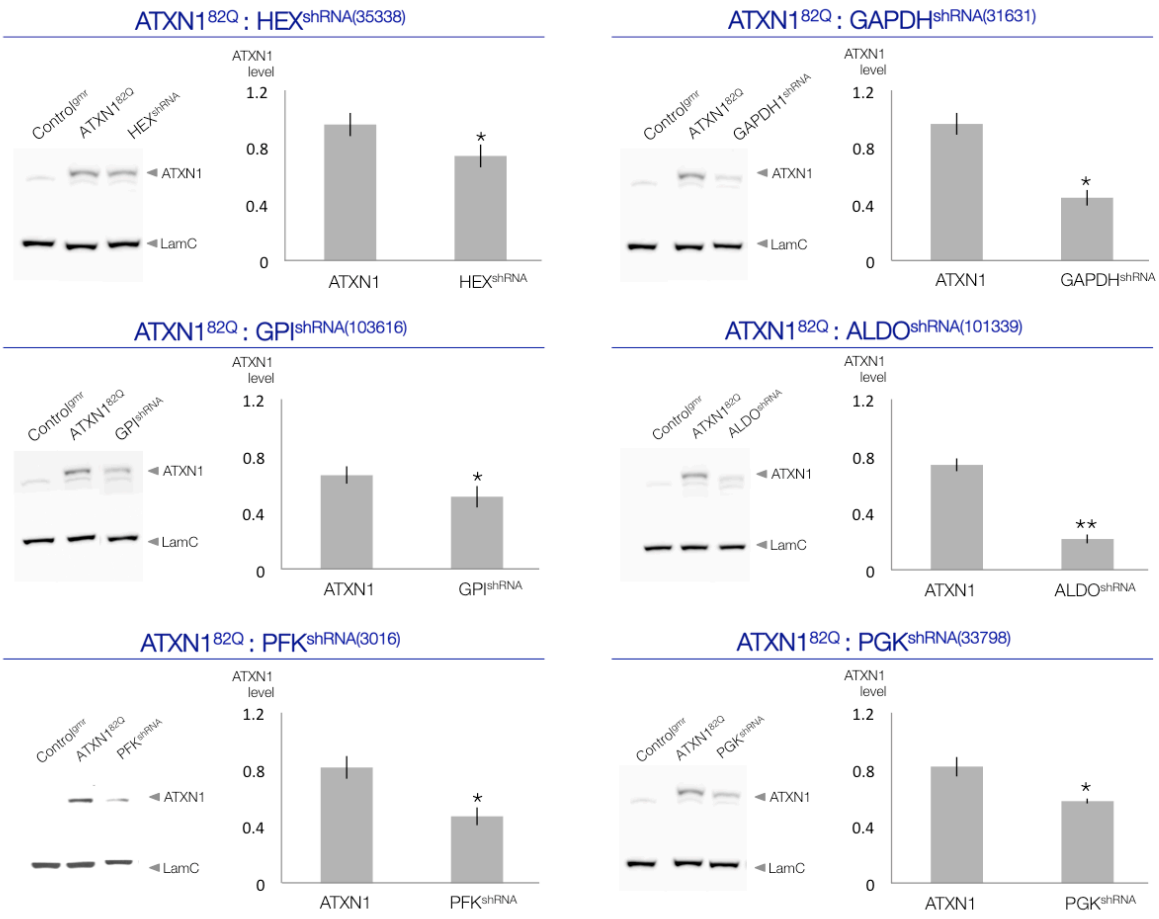


Figure 21. Knock-down of genes involved in glycolysis leads to a decrease of ATXN1^{82Q} protein levels in flies. Decreased levels of ATXN1^{82Q} protein induced by decreased levels of *Drosophila* homologues of the HEX, GPI, PFK, GAPDH, ALDO and PGK using shRNA by western blots (WB). Driver: gmr:GAL4. All WB were made in triplicate. Statistics: T- test *p<0.05, **p<0.005.

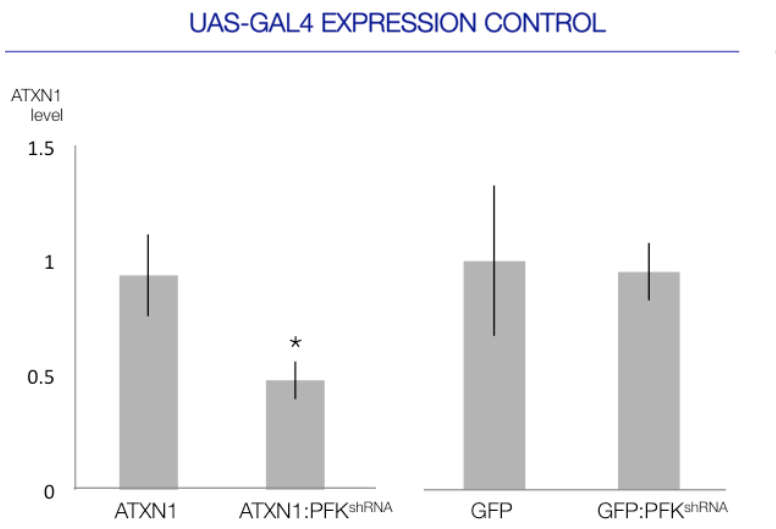


Figure 22. PFK knock-down decreases ATXN1^{82Q} protein levels and does not impact expression of a GFP reporter from the UAS-GAL4 system. On the left, PFK loss of function allele increases ATXN1 protein levels. On the right, PFK loss of function allele does not impact in GFP protein levels. Measured by Western Blot. Driver: gmr:GAL4. All WB were made in triplicate. Statistic: T- test *p<0.05.

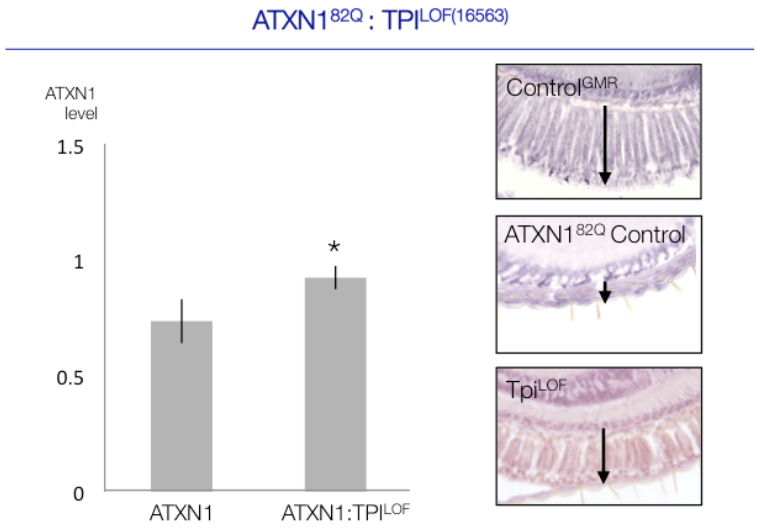


Figure 23 Loss of function allele of the *Drosophila* homolog of TPI increases ATXN1^{82Q} protein levels and suppresses eye retina phenotype. On the left, TPI loss of function allele increases ATXN1 protein levels measured by western blot. On the right, TPI loss of function allele suppresses retina eye phenotype caused by ATXN1^{82Q} expression. Driver: gmr:GAL4. WB was made in triplicate. Statistic: T- test *p<0.05.

DISCUSSION

5. DISCUSSION

5.1. Analysis of glucose uptake in the central nervous systems of mouse and *Drosophila* SCA1 models

Previous studies pointed to decreased insulin sensitivity and insulin resistance in SCA1 patients (Lalić 2010). The activation of INSR triggers the translocation of glucose transporter to the membrane that mediates the facilitated influx of glucose (Schulingkamp 2000). Insulin resistance is defined where normal or elevated insulin level produce sensitivity impairment to insulin mediated glucose disposal (Wilcox 2005). Insulin resistance is often associated with a decrease in insulin-stimulated glucose import (Choi 2002). I hypothesize that SCA1 pathogenesis causes glucose import impairment. To test this hypothesis, I performed gene expression profile and glucose import studies in SCA1 animal models.

Expression of many genes changes as disease progresses in other neurodegenerative disorders (Dutta 2013, Saetre 2011). Since SCA1 is a progressive disorder, I studied gene expression profiles in the cerebellum of B05 ATXN1^{82Q} transgenic mice at two different time points. Transcriptomic analyses using informatics approaches in younger transgenic animals [five week old, when first behavioral deficits emerge (Burright 1995)] do not show activation/inhibition in glucose uptake compared to wild-type mice (Fig 16). However, I observe a reduction in the expression of Slc2a1 gene, which is a glucose transporter (Fig 16, Fig 17). Slc2a1 is expressed at highest levels in the endothelial cells of blood–brain barrier tissue in human adults, supplying glucose to the cells of the central nervous system (Takata 1990). This suggests that glucose uptake is downregulated from early stages in B05 SCA1 transgenic mice. In the case of older transgenic mice [12 week-old, when disease symptoms are more prominent (Burright 1995)], I found

4.5 times more glucose metabolism genes transcripts altered, compared with five-week-old transgenic mice (Fig 16). Thus, the number of glucose metabolism genes misregulated in SCA1 transgenic animals appears to correlate with progression of disease pathogenesis. Bioinformatic analysis predicts inhibition of glucose uptake (Fig 16). In addition, I found reduced expression in the Slc2a13 and InsR genes (Fig 16, Fig 17). Slc2a13 is a glucose transporter that is expressed in the cerebellum (Allen Mouse Brain Atlas). The InsR (insulin receptor) is activated by insulin, and then glucose transporters are translocated to the plasma membrane allowing glucose uptake (Schulingkamp 2000). In older (12-week-old) B05 ATXN1^{82Q} transgenic mice, I do not find downregulation of the expression of Slc2a1 transporter gene as observed in younger (5-week-old) B05 ATXN1^{82Q} transgenic mice. One possibility is that this difference is a consequence of the progression of the disease.

B05 ATXN1^{82Q} transgenic mice express mutant ATXN1 protein only in Purkinje cells (Burright 1995). Thus, transgenic mice show only the phenotype associated with dysfunctional Purkinje cells. In principle, knock-in mice better recapitulate the normal spatial and temporal expression patterns at endogenous levels (Watase 2002). In addition, ATXN1^{154Q} knock-in mice show a slower progression of the disease compared to B05 ATXN1^{82Q} transgenic mice (Watase 2002). Therefore, I investigated the gene expression profile of ATXN1^{154Q} knock-in mice at 8 weeks of age [mice already show behavioral deficits (Watase 2002)] to corroborate the results obtained with the transgenic one. In these mice I find that the expression level of Slc5a1, which is a sodium/glucose cotransporter, is downregulated, suggesting impairment in glucose import (Fig 16, Fig 17). Further, bioinformatic analysis predicts inhibition of glucose uptake (Fig 16).

To study glucose import impairment in neuronal cells *in vivo*, I performed PET imaging in B05 ATXN1^{82Q} transgenic mice. These mice were injected with fludeoxyglucose (FDG), a glucose analog that contains a radioactive isotope. FDG is imported to cells through glucose transporters where is converted to FDG

phosphorylated (FDG-P) by hexokinase. FDG-P cannot proceed further in the glycolysis pathway and therefore it accumulates in the cytoplasm. Since these animals only express ATXN1^{82Q} transgene in the Purkinje cells (Burright 1995), I study glucose import in the cerebellum. Transgenic mice cerebella show a decrease in radioactive signal compared to wild-type animals (Fig 15). This result supports our hypotheses, that glucose uptake is downregulated in SCA1 transgenic mice cerebellar tissue. I also measured radioactive emission using a gamma counter in the mouse cerebellum. Gamma counting analysis revealed that transgenic mice cerebellums show lower radioactive signal than wild-type mice (Fig 15).

Taken together, these data from SCA1 mice supports the idea of glucose uptake impairment as a pathogenic mechanism in the SCA1 cerebellum.

I also investigated if glucose metabolism is impaired in the *Drosophila* SCA1 model, which expresses ATXN1^{82Q} in all neurons. Gene expression analysis in 1-day-old flies predicts inhibition in glucose uptake in agreement with the SCA1 mouse data (Fig 16). No alterations were found in the expression of glucose transporters; however, glucose uptake in SCA1 flies may be impaired by another mechanism. Metabolomic (NMR) analysis in one-day-old flies expressing ATXN1^{82Q} in the nervous system reveals the reduction in glucose compared with wild-type flies (Fig 18). Glucose is the first substrate in the glycolytic pathway. I do not know if the glucose detected is intra or extracellular; therefore, I cannot conclude that glucose import is impaired in SCA1 flies. However I suggest that the pathogenesis induced by ATXN1^{82Q} causes glucose metabolism impairment in the *Drosophila* nervous system.

5.2. Analysis of glycolysis gene expression in mice and *Drosophila* SCA1 models

Previous reports established a connection between impairment in glucose transport and glycolytic flux inhibition (Choi 2002, Xin 2012). Therefore, I hypothesize that glucose uptake impairment leads glycolysis flux downregulation caused by SCA1. As described above, I sequenced the transcriptome of SCA1 transgenic mice (two different time points), SCA1 knock-in mice, and SCA1 transgenic flies.

B05 ATXN1^{82Q} transgenic mice with five weeks of age do not show glycolytic genes transcripts altered. However, older transgenic mice (12 week-old) show reduced levels of Hk2, AldoC, Gapdh, Eno1b and Pfkfb3 transcripts Bioinformatic analysis predicts inhibition of glucose uptake (Fig 17). Hk2 shows a very low expression level in mice cerebellum (Allen Mouse Brain Atlas) in contrast to other hexokinases. Hk2 catalyzes the first glycolytic reactions, which is irreversible. The other 4 genes (AldoC, Gapdh, Eno1b and Pfkfb3) show mild/high expression levels in mice cerebellum (Allen Mouse Brain Atlas). AldoC, Gapdh and Eno1b enzymes catalyze the steps, four, six and nine respectively, which are reversible reactions (Berg, 2002). On the other hand, Pfkfb3 enzyme controls glycolytic flux by determining the intracellular concentration of fructose-2,6-bisphosphate, an allosteric activator of the glycolytic enzyme PFK (Van Schaftingen 1981, Yang 2014). These data suggest that expression of glycolytic genes is progressively reduced in transgenic mice compared to wild type as disease progresses.

Previous mass spectrometry analysis reported G6P reduction in 14-week-old ATXN1^{154Q} knock-in mice (Perroud 2013). G6P is produced by phosphorylation of glucose during the first step in the glycolysis pathway (Berg, 2002). This reaction is irreversible and is catalyzed by the enzyme hexokinase (Berg, 2002). This observation suggests a reduction of glycolysis in this mouse model.

RNA sequencing in 8-week-old ATXN1^{154Q} knock-in mice show that AldoC glycolytic gene expression is reduced (Fig 17). AldoC was also found downregulated in B05 SCA1^{82Q} transgenic mice. Since reduction in G6P was found in older ATXN1^{154Q} knock-in mice (14 weeks-old) it is possible that reduced expression of other glycolytic genes may occur at later stages.

ATXN1^{82Q} transgenic flies do no show any glycolytic gene expression alterations compared to WT flies. However, I found that PC and PCK1 human homolog expression are upregulated in SCA1 flies compared to wild-type. PC and PCK1 are two enzymes that play important roles in gluconeogenesis regulating two irreversible steps of this process (Jitrapakdee 2008, Xiong 2011). Gluconeogenesis is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate (Young 1977).

As described previously, metabolomics (NMR) analysis revealed reduction in glucose, maltose and pyruvate levels in SCA1 flies. Maltose is a disaccharide formed from two units of glucose that can be hydrolyzed into monosaccharaides and then enter the glycolysis (Berg 2002). The lower glucose and maltose levels found in SCA1 flies suggest that neurons are in a starvation-like condition that leads to the upregulation of gluconeogenic genes expression in certain tissues including the brain (Rothman 1991). In agreement with this, I found that expression of PC and PCK1 are increased. Pyruvate can be synthesized from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis. In gluconeogenesis, PC converts pyruvate to oxaloacetate, and then oxaloacetate is converted to phosphoenolpyruvate by PCK1 (Young 1977). Pyruvate levels are found decreased in SCA1 flies compare to wild-type (Fig 18). I suggest that decreased levels of glucose trigger downregulation of glycolytic flux resulting in pyruvate levels reduction. Moreover, pyruvate conversion to oxaloacetate by PC is increased, suggesting a decrease in pyruvate levels.

Together these data from SCA1 flies suggest that ATXN1^{82Q} in flies leads to downregulation of glycolysis and, in turn, this induces upregulation of gluconeogenesis.

5.3. Glycolysis as a modulator of SCA1 pathogenesis

The data described above support the idea that SCA1 pathogenesis leads to impairment of glycolysis in both SCA1 transgenic mice and flies. To evaluate the role of glycolysis in the toxicity caused by ATXN1^{82Q} in flies, I screened SCA1 flies with a knock-down (shRNA) of each glycolic gene using retinal degeneration as a readout. To drive expression of the ATXN1^{82Q} and shRNAs to the eye, I used the Glass Multimer Reporter (GMR) gene promoter, which is widely expressed in photoreceptor cells from third instar larval stage (Yu 2010).

In mammals, glycolytic reactions are mediated by the products of two or more redundant genes, with the exception of the reaction where GAPD is converted to 1,3PG, which is catalyzed by Gapdh (Berg, 2002). Mutations in one of the redundant genes may have a smaller effect on the fitness of the organism than expected from the gene function (Ardell 2001). However, each glycolytic reaction in *Drosophila* is catalyzed by one enzyme. Curiously, I also find an exception in flies in the same reaction catalyzed by Gapdh (Sun 1988). For this reaction, *Drosophila* has two genes encoding glyceraldehyde-3-phosphate dehydrogenase, Gapdh-1 and Gapdh-2. These two genes are highly expressed in larvae and late pupa, but not in adult, where the level of the Gapdh-1 transcript strongly decreases, whereas Gapdh-2 expression remains at a constant high level (Sun 1988). Therefore, using flies I minimize mechanisms that compensate for the loss of functional genetic information.

Although all these genes catalyze glycolytic reactions, only HK, PFK and PYK catalyze irreversible reactions. Consequently, each of them serves as a control site as well as catalytic roles (Berg 2002). The activities of these enzymes are regulated by covalent modification or by the reversible binding of allosteric effector. In addition, their amount is varied by the regulation of transcription (Berg, 2002). Here, I knockdown these genes using multiple "loss of function" mutations including shRNAs and P-elements inserted in genes locus disrupting its expression. shRNA *Drosophila* strains contain an inducible UAS-RNAi construct against a single protein-coding gene (http://stockcenter.vdrc.at.). I used shRNA transgene constructs that were selected for not having predicted off-targets (http://stockcenter.vdrc.at.). Thus, by means of these genetic tools that cause partial loss of function in the corresponding genes I expect to reduce the glycolytic flux.

I found that the retinal degeneration induced by SCA1 82Q transgene is ameliorated by knock-down of all the glycolytic genes including HK, PFK and PYK human homologs (Fig 19). I cannot predict if the GPI, ALDO, TPI, GAPDH, PGK, PGAM, ENO knockdown mutations lead to decreased glycolysis since all of these enzymes catalyze reversible reactions. However, knocking-down the HK, PFK and PYK homologs, I suppress histopathological features induced by ATXN1^{82Q} in the fly retina. These enzymes catalyze irreversible glycolytic reactions, thus, the knockdown of these three genes would be expected to reduce the glycolytic flux.

In conclusion, these data indicate that reducing glycolysis flux by knocking down glycolytic genes ameliorates toxicity induced by ATXN1^{82Q} in the *Drosophila* retina.

Next, I evaluated the effect of knocking down glycolytic enzymes in the pathogenesis caused by ATXN1^{82Q} in the nervous system using behavioral a readout. I drive the ATXN1^{82Q} expression to the nervous system inducing motor

performance deficits. In this case, I use nervana 2 gene (nrv2) promoter, which is expressed in central and peripheral nervous system (Sun 1995, Sun 1998), to drive the ATXN1^{82Q} expression. This assay allows us to study the progression of the SCA1 pathology in flies nervous system caused by ATXN1^{82Q}.

I use the same genetic tools (shRNA and P-elements insertions) that I used in the previous eye screen to induce partial loss of function of the corresponding genes in the nervous system. I found 7 mutations that correspond to 7 glycolytic genes (GPI, PFK, PGK, ALDO, TPI, GAPDH and PYK human homologs) that progressively improve the motor-performance phenotype showed by ATXN1^{82Q} flies (Fig 20).

However, I found four UAS-RNAi corresponding to four glycolytic genes (PGAM, GAPDH, PFK and ALDO) that enhance ATXN1^{82Q} fly motor performance phenotype. One possibility is that these UAS-RNAi constructs cause excessive loss of function of the corresponding genes leading to toxicity. To test this possibility, I examined more alleles from these genes using the retina and behavioral phenotypes. I found alleles from all these genes that suppress retina degeneration, and alleles from all these genes, except PGAM, that suppress behavioral deficits (Fig 19, Fig 20). Nevertheless, further controls should be done to evaluate it, such as analyzing the motor-performance phenotype of these UAS-RNAi constructs in a background with out the ATXN1^{82Q} transgene expression.

Together these data suggest that reducing the glycolysis flux improves the progressive behavioral deficits caused by ATXN1^{82Q} transgene expression in the *Drosophila* nervous system.

5.4. Mechanism to modulate of SCA1 pathogenesis

Identifying targets that can modulate the steady-state levels of a disease-causing protein is a key strategy to develop new therapeutic approaches since the severity of neurodegeneration in SCA1 correlates with the levels of the mutant ATXN1 protein. Proteasome and autophagy are two well-characterized mechanisms to reduce proteins levels (Kaushik 2010, Bhattacharyya 2014). Proteasome degrades damaged proteins by proteolysis and its impairment has been associated with SCA1 pathogenesis (Fernandez-Funez 2000, Bhattacharyya 2014). Autophagy is a mechanism of degradation and recycling of cellular components including proteins and organelles (Kaushik 2010). In the context of HD, activation of autophagy reduces mutant huntingtin accumulation leading to reduced toxicity (Ravikumar 2004). Thus, data from other neurodegenerative diseases suggest the potential role of autophagy to reduce levels of polyglutamine proteins such as ATXN1.

Low nutrient conditions lead to autophagy activation to generate energy through AMPK-mTORC1 signaling pathway inhibition (Rabinowitz 2010). Interestingly, a previous study showed that the inhibition of the glycolytic flux using the glycolysis inhibitor 2-DG leads to upregulation the proteasome activity under low ATP levels conditions (Huang 2010).

Metabolomic (NMR) analysis shows lower glucose and maltose levels in SCA1 flies nervous system compared to wild-type controls (Fig 18). In addition, transcriptomic analysis suggests that gluconeogenesis enzymes are upregulated in the nervous system of SCA1 flies (Fig 17). These data suggest that expression of $ATXN1^{82Q}$ in flies causes low nutrients-like condition. I hypothesize that SCA1 pathology decreases glycolytic flux as a compensatory mechanism to activate the protein degradation machinery such as autophagy and proteasome.

To test this hypothesis I study ATXN1 steady-state levels in SCA1 transgenic flies when I knockdown glycolytic genes. I found that reducing levels of the of HEX, PGI, PFK, ALD, PGK, and GAPDH *Drosophila* homologs leads to decrease in ATXN1 protein levels (Fig 21). As I have previously described, HEX and PFK work as control sites in glycolysis (Berg, 2002).

An exception is a TPI p-element LOF mutation increases $ATXN1^{82Q}$ levels (Fig 23). This mutation was also showed to suppress eye phenotype in SCA1 flies leading an increment in retina thickness (Fig 23). This suggests that $ATXN1^{82Q}$ protein levels may increase due to the increment in the size of the retina. Additional experiments using a loading control that reflects retinal size will clarify this point.

Future studies need to be done to evaluate if the glycolysis inhibition leads to reduce ATXN1 protein levels through the proteasome and/or autophagy activation.

In addition, I will study if the inhibition of the glycolysis in SCA1 mice triggers a decrease in mutant ATXN1 protein levels. I can use multiple approaches to inhibit glycolysis including glycolytic inhibitors that cross the brain blood barrier such as 2-DG. As well, I can decrease the glycolysis flux using transgenic mice that knock-down / knock-out the expression on glycolytic genes. I will measure ATXN1 protein levels under glycolysis inhibition context. Further, I will perform behavioral test to study if the inhibition of the glycolysis in SCA1 mice leads to decrease toxicity caused by the expression of mutant ATXN1.

CONCLUSIONS

6. CONCLUSIONS

- SCA1 pathogenesis causes glucose uptake impairment in SCA1 mice cerebellar cells.

- SCA1 pathogenesis causes glucose metabolism impairment in the *Drosophila* nervous system.

- Expression of glycolytic genes is progressively reduced in SCA1 transgenic mice compared to wild type as disease progresses.

- Downregulation of glycolysis caused by the expression of $ATXN1^{82Q}$ transgene in flies, in turn, induces upregulation of gluconeogenesis.

- Knocking down glycolytic genes in *Drosophila* ameliorates neurodegeneration by reducing steady-state levels of mutant ATXN1 protein.

Downregulation of glycolysis is a compensatory mechanism taking place in nervous system during SCA1 pathogenesis, suggesting new therapeutic approaches to decrease ATXN1 neurotoxicity

CONCLUSIONES

7. CONCLUSIONES

- La patogenia de SCA1 causa deficiencias en el transporte de glucosa en células del cerebelo de ratones.

- La patogenia de SCA1 causa deficiencias en el metabolismo de la glucosa en el sistema nervioso de *Drosophila*.

- La expresión de las enzimas glicolíticas se encuentra reducida en ratones transgénicos de SCA1. Está reducción se produce de manera progresiva de acuerdo con la progresión de la enfermedad.

- La disminución del flujo glicolítico por la expresión del transgen ATXN1^{82Q} en *Drosophila* induce el aumento de la gluconeogénesis.

- La inhibición parcial de la glicólisis en *Drosophila*, suprime la toxicidad que causa SCA1 en la retina y mejora los déficits comportamentales causados por la expresión de ATXN1^{82Q}.

- La disminución de la patogenia en *Drosophila* se debe a la reducción de los niveles de la proteína ATXN1.

La inhibición de la glicolisis es un mecanismo compensatorio que tiene lugar en el sistema nervioso como consecuencia de la patogenia de SCA1 abriendo la puerta a nuevos avances terapéuticos para disminuir la neurotoxicidad producida por ATXN1.

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